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TITLE: Summer Undergraduate Breast Cancer Research Program

PRINCIPAL INVESTIGATOR: William R. Folk, Ph.D.
Linda Blockus, Ph.D.

CONTRACTING ORGANIZATION: University of Missouri-Columbia
Columbia, Missouri 65211

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PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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6. AUTHOR(S)
William R. Folk, Ph.D.
Linda Blockus, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of Missouri-Columbia
Columbia, Missouri 65211

E-Mail: folkw@missouri.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

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13. ABSTRACT *(Maximum 200 Words)*

The Summer Undergraduate Breast Cancer Research Program (SUBCRP) at the University of Missouri-Columbia supported seven students in 2001.These students participated in faculty-mentored research projects for eight weeks and participated in seminars, brown-bag lunches, and specialty discussions on research, clinical trials, career opportunities, preparing for graduate school, communication skills, and ethics. The seven SUBCRP students joined the activities of the University's Life Sciences Undergraduate Research Opportunities Program, including 70 students from across the world involved in a wide variety of research experiences. The participating students included three African Americans, one Hispanic, and five females. Faculty from Biochemistry, Biological Sciences, Molecular Microbiology & Immunology, and Nursing served as mentors. Research projects included: 1) Sequence elements important for expression of urokinase plasminogen activator (uPA) in cancer cells; 2) An overview of post-breast cancer treatment for lymphedema; 3)Effectiveness of laser perometry for measuring limb volume in lymphedema; 4)Identification of Glc7 and its regulator Glc8 in Saccharomyces cerevisiae; Thermostability of murine Polyomavirus J domain mutants using circular dichroism; 6)Regulation of the putative estrogen receptor gamma by 17beta estadiol;7)Cross-resistance to chemotherapeutic drugs: Does resistance to one drug confer resistance to other drugs?

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INTRODUCTION

The Summer Undergraduate Breast Cancer Research Program (SUBCRP) at the University of Missouri-Columbia (MU) supported seven juniors and seniors in 2001. These students participated in faculty-mentored research projects for eight weeks and participated in seminars, brown-bag lunches, and specialty discussions on research, clinical trials, career opportunities, preparing for graduate school, communication skills, and ethics. The seven SUBCRP students joined the activities of the University's Life Sciences Undergraduate Research Opportunity Program, including 70 students from across the country involved in a wide variety of research experiences.

BODY

Recruitment and student selection

Information on the 2001 summer program was mailed to biology departments in Missouri and surrounding states, as well as other institutions that have a summer intern partnership with MU. Students were asked to provide a transcript, personal statement, resume and letter of recommendation. Applications were screened by the Project Director and Program Coordinator and then sent to potential faculty mentors for final selection/placement. University of Missouri-Columbia students applied for funding through the Life Sciences Undergraduate Research Opportunity Program (LS UROP). Their application included a transcript, two letters of recommendation, personal statement and a project proposal prepared with the guidance of their faculty mentor. Students were selected by faculty members of the LS UROP Advisory Committee.

A total of seven students were selected and funded in 2001, including three African Americans (2 female), one Hispanic female, and three white students (2 female). These students worked with six faculty members from five departments.

Research projects

Students worked in their research labs on a full-time basis for 8 weeks (June 11 - August 3, 2001) and received a scholarship. MU students received an amount consistent with the other LS UROP interns ($2400). Non-MU students received additional funding to off-set their living expenses. The student interns participated in weekly lab group meetings with their faculty mentor and other lab team members. On August 2, 2001, the 7 students funded by this grant participated in the Twelfth Annual MU Undergraduate Research Science Symposium and Luncheon. These students, along with over 70 other summer science interns, displayed posters describing their research projects, and received certificates at the awards luncheon. Invited guests included faculty mentors, lab members, campus administration, and the local media. Complete descriptions of the 2001 projects can be found in the abstract booklet for the Undergraduate Research Science Symposium. A list of the 2001 students funded on this grant, their home institutions, majors, hometowns, faculty mentors (and academic departments), and the research topics appear below.

Israel Collier, University of Missouri-St. Louis
Biology major from St. Louis, Missouri
Mentor: Dr. William Folk (Biochemistry)
Sequence elements important for expression of urokinase plasinogen activator (uPA) in cancer cells

Scott Culbertson, University of Missouri-Columbia
Biochemistry major from Blue Springs, Missouri
Mentor: Dr. Jane Armer (Nursing)
An overview of post-breast cancer treatment lymphedema

Julie Dusold, University of Missouri-Columbia
Interdisciplinary Studies major from Milwaukee, Wisconsin
Mentor: Dr. Jane Armer (Nursing)
Effectiveness of laser perometry for measuring limb volume

Michelle Harwerth, Southern Illinois University at Carbondale
Microbiology major from New Athens, Illinois
Mentor: Dr. Steven van Doren (Biochemistry)
Thermostability of murine Polyomavirus J Domain mutants using circular dichroism

Johanna Ortiz, College of St. Elizabeth (NJ)
Biology major from Jersey City, New Jersey
Mentor: Dr. John Cannon (Molecular Microbiology and Immunology)
Identification of Glc7 and its regulator Glc8 in Saccharomyces cerevisiae

Karyn Pleasant, Florida A&M University
Biology major from Oxon Hill, Maryland
Mentor: Dr. Dennis Lubahn (Biochemistry/Child Health)
Regulation of the putative estrogen receptor γ by 17β estradiol

Jarron Tilghman, University of Missouri-Columbia
Biology major from St. Peters, Missouri
Mentor: Dr. Stephen Alexander (Biological Sciences)
Cross-resistance to chemotherapeutic drugs: Does resistance to one drug confer resistance to other drugs?

Educational/Career Activities and Workshops

In addition to their research projects, interns participated in a variety of enrichment and social activities as part of the summer undergraduate research community at MU. The activities were organized and hosted by Dr. Joel Maruniak, Associate Professor of Biological Sciences, and Program Coordinator Dr. Linda Blockus. In addition to the 7 Breast Cancer interns, we had 20 MU students supported on University Funds, 13 students participating in the Plant Genomics Internship Program, and 10 NSF-REU interns that were regular participants in our educational and career activities. Our activities for 2001 included:

* Staff from the campus Environmental Health and Safety Office presented special workshops on lab safety, hazardous materials, and radioactive materials.

* Non-MU students were given a special tour of the main library by a science reference librarian.

* Three brown bag lunches provided an informal opportunity for students to present their projects in small groups to other science interns.

* Dr. Joel Maruniak and Dr. Linda Blockus presented a brown bag lunch workshop on 'Preparing your Abstract and Research Poster' in preparation for the Undergraduate Research Science Symposium.

* Dr. Silvia Jurisson (Radiopharmaceutical Chemistry) and Dr. Carol Ward (Biological Anthropology) were invited to a brown bag lunch to discuss 'Balancing a family and a career in science.'

* Dr. Jim Groccia, Director of the Program for Excellence in Teaching and of the MU Future Faculty Initiative discussed teaching careers at post-secondary institutions at a brown bag lunch.

Evening seminars included a number of topics on science, careers, resources, and ethics. Our topics for 2001 were:

'Finding your right livelihood' - Joel Maruniak, Biological Sciences

'Implications of Plant Genomics' - Georgia Davis, Agronomy

'Scientific Essentials for Research Students' - Karen Cone, Biological Sciences

'Web Resources' (NextWave, GrantsNet, Grad School Registry, PubMed, OVID) - Linda Blockus & Joel Maruniak, Biological Sciences

'Medical School Admissions' - Judy Nolke, MU School of Medicine

'Astrobiology and the search for life in the solar system' - Jack Burns, MU Vice Provost for Research

'Embryonic stem cell research' - Jason Meyer, Graduate Student in Biological Sciences

'The science of telomeres and life extension: The fountain of youth?' - Joel Maruniak, Biological Sciences

'Clinical Trials and Cancer Research' - Michael Perry, MD and Director of MU Hematology & Oncology

'Curiosity doesn't always kills the cat – Career reflections from a National Academy of Science member' - Mike Roberts, Veterinary Pathobiology/Animal Sciences

'Floral evolution in wild tobaccos: prying into the private lives of plants.' - Tim Holtsford, Biological Sciences

'Practicing science in for-profit and international arenas' - Jake Halliday, President & CEO of ABC Laboratories

'Applying to graduate programs in the life sciences' - Chris Hardin, Physiology & Dennis Lubahn, Biochemistry

'The switch from a university career to a high school learning environment' - Paul Mahoney, Columbia Independent School

'Science research and public policy: Testifying before Congress' - Fred vomSaal, Biological Sciences

New to our intern activities in 2001 were six specialty discussions organized by the Plant Genomic Internship program faculty (PGI) and the Breast Cancer program faculty (SUBCRP). Although organized for interns in those programs, attendance was open to all summer interns.

Speciality discussion topics included:

'Plant structural genomics: Mapping and genome analysis' - Jack Gardiner, Project Manager, NSF Maize Mapping Project

'At the Chasm Between Technology and Society: Rise of Research Consortia in Life Sciences' - Andrew Balas, MD/PhD and Director of the MU Center for Health Care Quality

'Plant functional genomics: Application of studies in yeast to understanding plant gene function' - David Eide, Nutritional Sciences

'A comparative oncology approach to breast cancer research' - Carolyn Henry, Veterinary Oncology

'Genetic engineering in crops: The GMO debate' - Georgia Davis, Agronomy and Karen Cone, Biological Sciences

'Clinic research trials for breast cancer' - Lisa Jacobs, Surgical Oncology

Students were offered an opportunity to participate in two scientific field trips: (1) to the Washington University Genome Sequencing Center and Monsanto's Plant and Biotechnology Research campus; and (2) to the Shaw Research Center, which is part of the Missouri Botanical Garden for a tour and presentation about MU's Center for Phytonutrient & Phytochemical Studies and collaboration with the Shaw Research Center.

Five of our educational activities in 2001 were a direct result of the addition of the U.S. Army funded breast cancer internships to our collection of undergraduate programs. These speakers (Perry, Balas, Henry and Jacobs) and the field trip to the Shaw Research Center were made available to all undergraduates, thus increasing the impact of our program well beyond the seven students specifically funded by this grant.

Assessment and Evaluation

Summer interns and faculty participated in the on-going efforts of the LS UROP office to determine the impact of summer research internships and activities and to improve faculty mentoring skills. Students were asked to complete two "critical incident reports" to provide insight into what events during the program have been most important to their consideration of a career in science. Coding of the open-ended responses indicates that there are both negative and positive events, and that speakers and the poster session play an important role along with the actual laboratory experience. Data is currently being analyzed to determine if pre-graduate students differ from pre-medical students in the types of events that have the most impact. Summer interns are asked to complete the "Confidence in Inquiry-related Skills" survey at the beginning and end of the summer program. In addition to comparing the pre/post scores of intern confidence on 20 scientific research skills, intern scores are also compared with their faculty mentor's assessment for each skill at the end of the program. Preliminary analysis of data collected in 2001 indicates some interesting results. Data will be collected again in 2002, and we anticipate being able to use this instrument with collaborators at other institutions for summer programs in 2003. Surveys administered at the end of the summer to interns and faculty mentors request information on the quality, quantity, content and method of communication that the student has with the mentor and others in his/her lab. This data will be linked to items in the "Confidence in Inquiry-related Skills" and used to provide faculty mentors as a group with feedback. LS UROP also maintains a student database of previous interns (currently over 900 students since1989) with educational and career information for longitudinal tracking. The

database also contains information on student publications and poster presentations. Alumni are contacted periodically to update their file with graduate degrees earned and career information.

KEY RESEARCH ACCOMPLISHMENTS

The primary purpose of this project is to provide a research experience for undergraduates. As such, any significant results of their research projects would be incorporated as preliminary data into the on-going activities of their faculty mentor's laboratory.

REPORTABLE OUTCOMES

1) We will be contacting the Summer 2001 interns this summer (2002) to determine if they have completed their bachelor's degree, entered graduate/professional school, and co-authored any additional publications or presentations.

2) Summer intern Jarron Tilghman presented his 2001 summer poster this past November (2001) at the *Annual Biomedical Research Conference for Minority Students* in Orlando, Florida. This conference is sponsored by the Division of Minority Opportunities in Research, National Institute of General Medical Sciences.

3) Data collected from students and faculty as part of the larger LS UROP assessment and evaluation project is still being analyzed for results and eventual publication.

CONCLUSIONS

We were pleased with the student participation and faculty mentoring for our first Summer Undergraduate Breast Cancer Research Program. Minor adjustments in logistical arrangements and recruiting were made for the 2002 summer. Seven students are being supported by this program in 2002 (three from MU, four from other institutions). Because of the increased visibility of breast cancer undergraduate research opportunities on campus due to the U.S. Army program, an additional three MU students are conducting cancer-related research this summer (2002) and are being funded through other sources. We will again include cancer research seminars in our list of educational activities and invite all undergraduate researchers (upwards of 70) to attend.

REFERENCES

None.

APPENDICES

One copy of the abstract book for the 12th Annual Undergraduate Research Science Symposium has been sent along with this annual report.

University of Missouri - Columbia

12th Annual Undergraduate Research Science Symposium

1:30 - 3:30 p.m.

Thursday, August 2, 2001

Donald W. Reynolds Alumni Center

University of Missouri – Columbia

Twelfth Annual
Undergraduate Research Science Symposium

Thursday, August 2, 2001
Donald W. Reynolds Alumni Center
1:30 – 3:30 p.m.

Abstracts in this book describe the scientific research projects of over eighty-five students in summer research internship programs sponsored by the University of Missouri-Columbia.

Participating Research Departments and Divisions

Agronomy
Animal Sciences
Biochemistry
Biological Engineering
Biological Sciences
Cardiothoracic Surgery
Chemistry
Child Health
Dalton Cardiovascular Research Center
Mechanical & Aerospace Engineering
Molecular Microbiology & Immunology
Nuclear Engineering
Nursing
Nutritional Sciences
Orthopaedic Surgery
Physics & Astronomy
Physiology
Plant Microbiology & Pathology
MU Research Reactor
Veterinary Biomedical Sciences
Veterinary Pathobiology
Physics, UMKC
Biochemistry, UMR
Civil Engineering, UMR

Abstract Book Prepared by:
LS UROP Office
422 Tucker Hall
University of Missouri-Columbia
Columbia, MO 65201-7400
(573) 882-5979
www.lsurop.missouri.edu

Symposium Web Site and On-Line Abstracts Created by Ramona Fairchild and Alan Marshall, Division of Biological Sciences.

Financial Support and Program Backgrounds

Life Sciences Undergraduate Research Opportunities Program (LS UROP)

The Summer 2001 LS UROP Undergraduate Research Internships are funded by the MU Office of the Provost. Twenty MU juniors and seniors conducted an independent research project in the life sciences under the guidance of an MU faculty member to experience research first-hand and prepare the students for biomedical, scientific and teaching careers. Students attend evening seminars and brown bag lunches to discuss issues related to scientific research, including ethics, career paths, emerging research areas, and opportunities for post-baccalaureate study. Administrative support for the LS UROP office, which is the successor of the MU Hughes Program (1989-1998), is funded by Life Science Mission Enhancement funds. The LS UROP office also coordinates several other programs.

LS UROP Advisory Board:
Dr. Karen Bennett, Molecular Microbiology and Immunology
Dr. Karen Cone, Biological Sciences
Dr. John David, Chair, Biological Sciences
Dr. James Carrel, Biological Sciences
Dr. Kevin Fritsche, Animal Sciences
Dr. Meredith Hay, Veterinary Biomedical Sciences
Dr. Virginia Huxley, Physiology
Dr. Ray Semlitsch, Biological Sciences
Dr. James Spain, College of Agriculture, Food & Natural Resources
Dr. Roger Sunde, Nutritional Sciences
Dr. Henry White, Physics and Astronomy
Dr. Warren Zahler, Biochemistry

Program Coordinator:	Dr. Linda Blockus
Summer Seminar Coordinator:	Dr. Joel Maruniak
Administrative Assistant:	Suzy Otto
Fiscal Assistance:	Jaime Campbell, Tyeece Little, Pat Willis
Graduate Students:	Jenelle Frost, David Giblin, Erin Morris

National Science Foundation - Research Experience for Undergraduates (Life Sciences)

Currently in the second year of another multi-year grant from NSF, this program supports undergraduates from several regional and historically black colleges and universities to conduct independent research projects with faculty at MU. Students selected by the cooperating institutions participate in an eight week summer research program in the areas of cell, molecular, and genetic biology. Students attend educational enrichment activities with the LS UROP Interns throughout the summer. Continuing partnerships have been established with Florida A&M University, Grinnell College, Long Island University-Brooklyn campus, Prairie View A&M University, Southwest Missouri State University, Truman State University, University of Arkansas-Pine Bluff, and Xavier University of Louisiana. The Division of Biological Sciences and LS UROP have received continuous support from the NSF-REU program since 1991.

Principle Investigator:	Dr. John David, Biological Sciences
Co-PI:	Dr. Linda Blockus, LS UROP

National Science Foundation - Research Experience for Undergraduates (MU Research Reactor)

The Research Reactor Center of the University of Missouri (MURR) has won competitive NSF grant funding for undergraduate research continuously since 1989. Ten students (or more when additional funding can be obtained) are selected each year from responses to nationwide advertisement. The students are paired with MURR/UMC researchers to pursue a ten-week summer research project. The MURR-REU program meets regularly for required radiation training and for lecture/discussion sessions covering research methods, topics, interests, ethics and interaction with news media. Additional internships are supported for journalism/media students to participate alongside their scientific peers. This experience helps all students better appreciate the difficulties of science/media communications and the need for improving science literacy for the general public.

Program Director: Dr. Fred Ross, Physics and Astronomy

Plant Genomics Internships at MU

MU has long been nationally recognized as a center for plant genetics research. The purpose of the Plant Genomics Internships at MU (PGI@MU) is to demonstrate the excitement and career options available in the field of plant genomics research to talented undergraduates. Fifteen faculty from seven research units, representing ten projects funded through the NSF Plant Genome Research Program serve as mentors for this program. Thirteen students from eleven institutions were selected for PGI funding for 2001. In addition to attending evening seminars and brown bag lunches with the other summer interns, PGI students participated in specialty presentations on plant genomic topics and toured the Washington University Genome Sequencing Center and the plant and biotechnology research facilities at Monsanto. The MU plant genomic faculty, in collaboration with LS UROP, have secured a three-year, $191,000 grant from the National Science Foundation to fund this summer program.

Program Director: Dr. Karen Cone, Biological Sciences

Summer Undergraduate Breast Cancer Research Program

Over twenty-five externally funded breast-cancer research projects are currently underway under the guidance of MU faculty in five colleges and schools. The Summer Undergraduate Breast Cancer Research Program provides a mentored research internship experience related to breast cancer for up to eight students (half from MU, half from other institutions). In addition to evening seminars and brown bag lunches with other summer interns, breast cancer interns participated in three specialty presentations on clinical research. Support for this program comes from a three-year, $129,500 competitive grant awarded to MU from the U.S. Army Medical Research and Material Command specifically for undergraduate research training. This program is coordinated by the LS UROP office.

Priniciple Investigator: Dr. William Folk, Biochemistry
Co-PI: Dr. Linda Blockus, LS UROP

Missouri Ozark Forest Ecosystem Project - Internships in Avian Ecology

The Missouri Ozark Forest Ecosystem Project (MOFEP) is a multi-dimensional experiment examining the effects of two timber harvest techniques on the Ozark ecosystem. This year was the fifth following timber removal, which was done in 1996. Funding for the avian research component comes from the Missouri Department of Conservation. Sixteen interns from across the U.S., Mexico and the Dominican Republic spent 10 weeks working in the Missouri Ozarks, with early efforts spent spot-mapping bird locations and finding and monitoring bird nests. Since late June, interns have been mist-netting and banding birds and working on individual research projects. Individual projects shown at the poster session involve all aspects of Ozark ecology and are completed while the students are still doing most of their normal duties for MOFEP.

Program Directors:	Dr. John Faaborg, Biological Sciences, MU Dr. Paul Porneluzi, Central Methodist College Richard L. Clawson, Missouri Department of Conservation
Graduate Supervisors:	Rafael Brito Aguilar, Biological Sciences Dana Morris-Porneluzi, Biological Sciences

F.B. Miller Undergraduate Summer Research Program in Animal Sciences

The Miller Undergraduate Summer Research Program prepares undergraduates for graduate study in Animal Sciences. An advisory committee selected four students to receive a stipend and conduct research with faculty mentors for the areas in which they hope to pursue graduate study. Interns learn about graduate school life, advanced library and computing skills and effective ways to present their work. Upon conclusion of their research internships at MU, interns make formal presentations of their research to faculty and peers at the Undergraduate Research Symposium and submit a paper summarizing their work. Financial support is provided by the F.B. Miller Fund which is an endowment to the Animal Sciences Department in support of research, scholarships and development of livestock in Missouri.

Program Director:	Dr. Matt Lucy, Animal Sciences
Staff Assistant:	Cindy Glascock

National Science Foundation Access to Doctoral Education (ADE)

Missouri's Alliance for Graduate Education and the Professoriate (MAGEP) Access to Doctoral Education (ADE) Summer Research Program is sponsored by the National Science Foundation, and introduces rising minority juniors and seniors majoring in Science, Engineering, and Mathematics (SEM) fields to career opportunities in academia. The program includes a mentored research experience, GRE preparations, workshops about funding sources for graduate education, as well as seminars on how to succeed in graduate school.

Principal Investigator:	Dr. Charles Sampson, Graduate School
Program Director:	Dr. Lenell Allen
Administrative Assistant:	Bev Vaughan
Graduate Assistant:	Olga Bolden-Tiller

Students Participating in the Undergraduate Research Science Symposium

August 2, 2001

Life Sciences Undergraduate Research Opportunities Program (LS UROP)

Malinda Boyd, University of Missouri - Columbia
Amy Chamberlain, University of Missouri - Columbia
Jeremy Cravens, University of Missouri - Columbia
Brett Emo, University of Missouri - Columbia
Dirk Erickson, University of Missouri - Columbia
Krista Fohey, University of Missouri - Columbia
Darren Gentry, University of Missouri - Columbia
Lauren Hart, University of Missouri - Columbia
Michael Hughes, University of Missouri - Columbia
Julie Janes, University of Missouri - Columbia
Matt John, University of Missouri - Columbia
Katie Konrad, University of Missouri - Columbia
Dana Lambert, University of Missouri - Columbia
Danny Liu, University of Missouri - Columbia
Brandi Schottel, University of Missouri - Columbia
Aaron Tesfai, University of Missouri - Columbia
Rachel Walsh, University of Missouri - Columbia
Christopher Wheatley, University of Missouri - Columbia
Andrew Wheeler, University of Missouri - Columbia
Karen Williams, University of Missouri - Columbia

National Science Foundation - Research Experience for Undergraduates (Life Sciences)

Stacey Baptiste, Long Island University - Brooklyn Campus
Kevin Berry, Grinnell College
John Bisges, Truman State University
Brittainy Dark, Florida A&M University
Kelli Dixon, University of Arkansas at Pine Bluff
Regina Hall, Xavier University of Louisiana
Charcacia Sanders, Prairie View A&M University
Kimberly Sartain, Southwest Missouri State University
Charlotte Schnellbacher, Truman State University
Taiya Williams, Prairie View A&M University

National Science Foundation - Research Experience for Undergraduates (MU Research Reactor)

Ethan Balkin, University of Missouri - Columbia
Ryan Dotson, University of Missouri - Columbia
Joseph Haney, University of Missouri - Columbia
Mark Patty, University of Missouri - Columbia

Plant Genomics Internships at MU

Craig Barrett, Hartwick College
Pamela Conerly, Mississippi State University
Catherine Cupples, University of Missouri - Rolla
Courtney Hoshibata, Harvey Mudd College
Kristen Leach, Texas A&M University
Timothy Pouland, Western State College of Colorado
Thomas Ream, University of Missouri - Columbia
Katharine Swoboda, University of Nebraska - Lincoln
Karen (Riddle) Trainor, Columbia College
Sarah Warren, Iowa State University
Timothy Wertin, University of Missouri - Columbia
Christopher Wilson, Hamline University
Dana Woodruff, University of Missouri - Columbia

Summer Undergraduate Breast Cancer Research Program

Israel Collier, University of Missouri - St. Louis
Scott Culbertson, University of Missouri - Columbia
Julie Dusold, University of Missouri - Columbia
Michelle Harwerth, Southern Illinois University at Carbondale
Johanna Ortiz, College of St. Elizabeth
Karyn Pleasant, Florida A&M University
Jarron Tilghman, University of Missouri - Columbia

Missouri Ozark Forest Ecosystem Project - Internships in Avian Ecology

Marie Abbott, James Madison University
Teresa Alfaro Reyna, Universidad Autonoma de Tamaulipas, Mexico
Lesley Avery, University of Missouri - Columbia
Derek Freund, University of Missouri - Columbia
Rafael Garcia Perez, Universidad Autonoma de Tamaulipas, Mexico
Sarah Grote, Central Methodist College
Salvador Luna Garcia, Universidad Autonoma de Tamaulipas, Mexico
Alejandra Martinez Cardenas, Universidad Autonoma de Tamaulipas, Mexico
Danilo Mejia, Grupo Ecologistas Tinglar, Dominican Republic
Venicio Mejia, Grupo Ecologistas Tinglar, Dominican Republic
Logan Nichols, Southeast Missouri State University
Darren Oakley, University of Missouri - Columbia
Shelley Pasternak, University of Missouri - Columbia
Garrett Rock, University of Missouri - Columbia
Teresa Sharillo, University of Connecticut
Laura Sullivan, Northeast Oklahoma State University

F.B. Miller Undergraduate Summer Research Program in Animal Sciences

Tesha Alston, St. Cloud State University
Melissa Hansen, Clemson University
Armitra Jackson, University of Arkansas at Pine Bluff
Doug Snider, University of Missouri - Columbia

National Science Foundation Access to Doctoral Education (ADE)

Richard Clemon, University of Missouri - Rolla
Anjuli Dahia, University of Missouri - Columbia
Timothy Dryer, Longview Community College
Karina Gilpin, University of Missouri - Columbia
Stacey Harley, University of Maryland Eastern Shore
Kandis Ingram, University of Missouri - Columbia
Latevi Lawson, Xavier University of Louisiana
Renita Oko, University of Missouri - Rolla
Maurice Penny, University of Missouri - Columbia
Mario Saldana-Torres, Polytechnic University of Puerto Rico
Saraia Smith, SLCC - Florissant Valley

Students Funded by Other Sources

Luciana Armilio, University of Missouri - Columbia, Department of Psysiology
Lindsey Atkinson, University of Missouri - Columbia, A&S Undergraduate Research Mentor Program
Regan Barnes, University of Missouri - Columbia, NSF-REU Supplement to K. Bennett
Summer Chaudhari, University of Missouri - Columbia, Academic Year LS UROP
Natalie Gooden, University of Missouri - Columbia, Undergraduate Research Mentorship Program in Nursing
Leslie Grill, University of Missouri - Columbia, Academic Year LS UROP
Chirag Patel, University of Missouri - Columbia, grant to T. Phillips
John Patterson, University of Missouri - Columbia, Academic Year LS UROP
Nicole Schultz, University of Missouri - Columbia, McNair Scholars Program
Darla Tharp, University of Missouri - Columbia, McNair Scholars Program

Marie Abbott

Hometown: Clifton, Virginia

Major: Biology

University: James Madison University

Faculty Mentor: Dr. John Faaborg and Dr. Paul Porneluzi, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Use of Clearcuts by Forest Breeding Bird Species

Marie T. E. Abbott and Teresa C. Sharillo

The purpose of this data analysis is to determine whether forest breeding bird species are using clearcuts, which are a type of timber harvesting forest treatment. The importance of this analysis is to see if clearcutting, which is a loss of breeding habitat for forest breeding birds, serves other purposes for these species. To conduct this analysis we used mist-netting data that sampled the bird species within and around the clearcuts during the month of July. To determine whether forest birds were defending territories in the clearcuts we used data from spot maps collected in late May through June. We sampled thirteen clearcuts of varying size, ranging from 9.3 hectares to 0.772 hectares, in Southeastern Missouri that were harvested in 1996. From these data we determined the number and age of forest species found in the clearcuts. We concluded from the analysis that the forest birds are using the clearcuts during the month of July but were not defending territories in late May and June. We also found that many of the forest breeding species caught in the mist-nets were hatch year birds, suggesting that the most likely use of the clearcuts for forest species is to protect their fledged offspring from predators and to utilize a more abundant food supply.

Tesha Alston

Hometown: Minneapolis, Minnesota

Major: Biomedical Sciences

University: St. Cloud State University

Faculty Mentor: Dr. Edmund Rucker, Animal Sciences

Funded by F.B Miller Undergraduate Research Program in Animal Sciences

Determination of transgenic copy number in the WAP-Bax transgenic mouse by TaqMan analysis

Tesha Alston, David Durtschi, and Edmund Rucker

Transgenic mice expressing the protein Bax under the WAP (whey acidic protein) promoter has been utilized to evaluate how Bax affects mouse mammary gland development. Previous research reveals that expression of this cell death protein should elevate apoptosis in mammary epithelia , thus causing a lactation defect in female mice. Currently we have 5 WAP-Bax transgenic lines that are being examined for Bax expression. The WAP-Bax transgene was introduced into mouse genome by pronuclear injection. To characterize the resulting transgenic mice there are two issues to address: 1) determining where the transgene has integrated and 2) determining the copy number of the transgene. Two methods by which the latter problem will be resolved are by TaqMan real-time PCR and Southern blot hybridization.

TaqMan real-time PCR uses a dual fluorogenic probe to detect accumulation of PCR product. During the extension phase, Taq polymerase cleaves the probe causing the reporter to be separated from the quencher. The reporter dye emits a detectable flourescent signal, thereby creating a direct relationship between emission intensity and PCR product. The Southern blot hybridization method is used to analyze transgene copy number by hybridizing genomic DNA to a radioactive DNA probe . The genomic DNA and the radioactive DNA is then visualized using autoradiography. Both techniques will be evaluated for sensitivity, reliabilty, and precision. It is expected the TaqMan real-time PCR will be a faster and more reliable, non-radioactive method used to evaluate the WAP-Bax transgenic mice .

Luciana M. Armilio

Hometown: Freeman, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Kerry S. McDonald, Physiology

Funded by Department of Physiology

Altered cardiac myofibrillar regulatory proteins in diabetic dyslipidemic pigs

Luciana M. Armilio, F. Steven Korte, Eric Mokelke, Michael Sturek, and Kerry S. McDonald

Chronic diabetes is often associated with cardiomyopathy or depressed heart function. This depressed heart function is suggestive of a defect in cardiac muscle proteins. The goal of this research was to investigate whether cardiac regulatory proteins are altered in a porcine model of diabetes. We first examined whether the isoform expression of the regulatory protein cardiac troponin T (cTnT) was altered with diabetic dyslipidemic pigs. Cardiac muscle protein samples were subjected to SDS-PAGE with subsequent western blotting using an antibody specific for cTnT. cTnT isoforms appeared to be shifted to greater expression of cTnT2 in hearts from diabetic dyslipidemic pigs. We also investigated whether cAMP dependent protein kinase (PKA) and protein kinase C (PKC) induced phosphorylation levels of myofibrillar proteins were altered in response to diabetic dyslipidemia. Baseline levels of myofibrillar phosphorylation were assessed by "back-phosphorylation" assays using radiolabelled phosphate. Autoradiograms showed that PKA phosphorylated the cardiac regulatory proteins myosin binding protein-C (MyBP-C) and troponin I (TnI) while PKC phosphorylated TnT in addition to MyBP-C and TnI. Baseline phosphorylation of MyBP-C and TnI by PKA appeared to be lower in diabetic dyslipidemic hearts, while preliminary results suggest that PKC induced basal phosphorylation is unchanged. Overall, these results indicate that some cardiac myofibrillar proteins are altered in pig hearts in response to diabetic dyslipidemia, and these changes may contribute to cardiac dysfunction associated with diabetic cardiomyopathy.

Lindsey Atkinson

Hometown: Kingdom City, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Mark Kirk, Biological Sciences

Funded by Arts and Science Undergraduate Research Mentor Program

Growth factors affecting neural regeneration in *Aplysia californica*

Lindsey Atkinson, Binaben Vanmali, Maria Messner, and Mark Kirk

Soluble and substrate-bound factors enhance neurite regeneration in cultures of bag cell neurons from the sea slug *Aplysia californica* . Bag cell neurons (BCNs) are a homogenous population of cells, making them very useful for comparative studies of nerve outgrowth. We found that sheath cells dissociated from pleural visceral connectives or arterial cells dissociated from the anterior aorta, enhanced neurite growth of BCNs, relative to controls. To determine whether the sheath and arterial cells are producing soluble growth factors, the substrate remaining after sheath and arterial cells were killed was tested for bioactivity. BCNs cultured on coverslips coated with the substrates, remaining after sheath and arterial cells were killed, exhibited enhanced growth when compared to that of live plates.

Nitric oxide was originally discovered as a soluble (gaseous) molecule that mediates dilation of arteries (Endothelium Derived Relaxing Factor, EDRF). Nitric oxide also mediates long term synaptic plasticity, which often involves growth of new nerve connections. Long-term synaptic plasticity underlies long-term potentiation at synapses, and thus affects learning and memory. Long-term potentiation is mediated by similar cellular mechanisms in vertebrate and invertebrate systems, so work on the sea slug can be applied to more advanced animal systems.

To test for the action of soluble growth factors, specifically nitric oxide, we applied the chemical L-NAME, a chemical that blocks nitric oxide production, to co-cultures of BCNs and arterial cells. We have shown that arterial cells elicit a statistically significant enhancement of neurite growth by BCNs when compared to BCN growth in the presence of L-NAME. These results suggest that nitric oxide is an important soluble component of the growth enhancing effects produced by arterial cells in co-cultures with BCNs.

Ethan R. Balkin

Hometown: Jefferson City, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. J. David Robertson, Chemistry

Funded by National Science Foundation - REU (MU Research Reactor)

Elemental Analysis of Consumer Market Nutriceuticals

Ethan R. Balkin and Dr. J. David Robertson

The herbal supplement or nutriceutical industry has grown remarkably in the past 6 years; with sales increasing from $8.6 billion in 1995, to $16 billion in 2000. It is estimated that greater than 50% of U.S. adults use dietary supplements. These materials fall under the Dietary Supplement Health and Education Act, and are not regulated by the FDA(or any other state agency)provided consumer labeling guidelines are followed.

The objective of this study was to screen the most popular herbal supplements for trace element content, and to determine if any elements are present in concentrations which will approach maximum tolerable daily intakes(MTDI'S). Given the wide range of elements and types of products commercially available, analysis by energy-dispersive x-ray fluorescence(ED-XRF) was determined to be the most efficient analytical method. ED-XRF can rapidly scan for 72 elements in 15 minutes with μg/g limits of detection for most elements. Using ED-XRF to analyze 44 supplements, we were able to identify 1 supplement which, when taken according to the manufacturers instructions, would easily exceed the MTDI level for chromium. Similarly, 24 supplements contain levels of lead which exceed the MTDI level for children under 6; 17 supplements contain lead levels which exceed the MTDI level for children over 7; and 4 supplements contain lead levels which exceed the MTDI level for pregnant women. Of the 44 supplements tested, chelaters and algal supplements were found to contain the highest levels of trace metals.

Stacey Baptiste

Hometown: Brooklyn, New York

Major: Biology

University: Long Island University-Brooklyn Campus

Faculty Mentor: Dr. Roger Sunde, Nutritional Sciences

Funded by National Science Foundation - REU (Life Sciences)

Glutathione peroxidase-1 activity in rodents

Stacey S. Baptiste, Sean M. Blake, Jacqueline K. Evenson, and Roger A. Sunde

We studied previously, the dietary effects of the trace element selenium on the activity of glutathione peroxidase-1 (GPX1). Studies in our laboratory have been done on rats, mice and avian species. The objective of this study was to quantitate and compare the activity of GPX1 in three rodents: hamsters, mice, and rats. The effect of dietary Se level on the activity of GPX1 was measured in rats fed a Se-deficient diet, or that diet supplemented with 0.02, 0.05, 0.075, 0.1, 0.15, 0.2 or 0.3 μg Se/g diet. Rat liver supernatant was assayed using the glutathione peroxidase coupled assay which measured the oxidation of NADPH to NADP+ at an absorbance of 340 nm. The Lowry protein assay was used to measure the amount of protein found in each sample and calculate the specific activity. GPX1 activity in liver supernatant ranged from 24.99+/-5.96 in Se-deficient rats to 765.30+/-15.04 EU/g protein in rats fed 0.2 μg Se/g diet. These values were significantly different at $p<0.05$. There were no significant differences in protein levels for any level of dietary Se.

In a second experiment, liver GPX1 activity in hamsters, mice, and rats (n=5) fed Se-adequate diets was measured. The results showed that liver supernatant GPX1 activity was 1326.58+/-64.17, 1023.44+/-58.48 and 773.76+/-102.68 EU/g protein for hamsters, mice and rats, respectively. Protein levels for hamsters and rats were not significantly different, however, mice were significantly lower (12.27+/-0.38, 12.19+/-0.25, and 10.37+/-0.29 mg/ml, respectively). In addition, plasma glutathione peroxidase (GPX3) enzyme activity in hamsters was one-fourth of the activity measured in mice and rats (15.64+/-2.55 versus 64.11+/-10.29 and 57.28+/-4.71 EU/g protein, respectively).

In conclusion, liver GPX1 enzyme activity is dramatically regulated by dietary Se. In the first experiment, significant differences were seen in rat liver GPX1 activities for different levels of dietary Se. Additionally, three different rodent species (hamster, mouse and rat) fed Se-adequate diets had significantly different liver GPX1 activities. The ranking order from highest to lowest liver GPX1 enzyme activity is hamster, mouse and rat. Whereas, the ranking for GPX3 activity from highest to lowest is mouse, rat and hamster.

Regan Barnes

Hometown: Marshalltown, Iowa

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Karen L. Bennett, Molecular Microbiology & Immunology

Funded by NSF-REU Supplement Grant to Faculty Mentor

Using reverse genetics to generate deletion mutants in germline RNA helicases and their interacters

Regan Barnes, April Orsborn, Ruth A. Montgomery, and Karen L. Bennett

Four germline-specific RNA helicase (*glh*) genes have been identified in the soil nematode *Caenorhabditis elegans* . It is predicted these RNA-binding proteins function to localize RNAs in the germ-line specific P granules. The specific role of the *glhs* is not yet known, but RNA interference studies suggests that they are critical for germline development. The Bennett laboratory is interested in learning about the function of the *glhs* as well as other interacters and the role they play in germline development.

In order to learn more about the *glhs* , we have attempted to isolate worms with mutations in the *glh* genes. To do this we expose the worms to 4,5,8-trimethylpsoralin (TMP), a mutagenizing agent that, when combined with UV light, causes DNA deletions. We then look at the DNA of the progeny (~500,000) of these mutagenized worms. By doing polymerase chain reactions using nested *glh* -specific oligonucleotide primers, we are able to see wildtype genes, and if present, mutants. Deletion bands that are smaller than the wildtype can be seen by imaging after gel electrophoresis. If there is a deletion, the next step in the process is to do sibling selection to isolate the progeny of the original worm with the deletion, eventually ending up with a plate of worms comprising the deletion strain.

In previous summers there have been deletions in two of the four *glhs* . Current work is now being done on them. As of this summer, we have been unsuccessful in deleting a *glh* gene. Because we are looking for the loss of ~2x 10^3 base pairs out of the $1x10^8$ base pairs in the genome, it may not be surprising we don't always succeed.

Craig F. Barrett

Hometown: Ilion, New York

Major: Biology

University: Hartwick College

Faculty Mentor: Dr. John Walker, Biological Sciences

Funded by Plant Genomics Internship at MU

Functional genomics of plant phosphorylation: Identification of T- DNA insertions in the type I protein phosphatases of *Arabidopsis thaliana*

Craig F. Barrett, Jason T. Doke, and John C. Walker

Protein phosphatases play key roles in regulating cellular processes of growth and development. Their purpose is the dephosphorylation of proteins, which in turn changes their function. In the genome of *Arabidopsis thaliana* , genes encoding at least eight different Type I serine/threonine protein phosphatases (*TOPP*) have been identified. Southern Blot Analysis was used to identify transfer DNA (T-DNA) insertions (from *Agrobacterium tumefasciens*) within *TOPP* genes in a population of transgenic plants, in concert with the Arabidopsis Knockout Facility. The screening process consisted of two consecutive rounds of Southern Blot Analysis. This was followed by identification of potential individual plants containing insertions. A first- round screen was done for *TOPP 8* , revealing ten "hits" in the superpool of plants containing individuals with insertions. For *TOPP 3* and *TOPP 5* , second- round screens identified subpools with insertions in their genes. First round screens for these two genes were previously done. An individual plant with an insertion was identified in *TOPP 4* . This plant was heterozygous and showed no modified phenotype. Self- fertilized seeds from this individual will be planted and the progeny analyzed to identify homozygous *TOPP4* loss- of- function mutants and any associated phenotype. This study will help contribute to a better understanding of the mechanisms of phosphorylation and dephosphorylation.

Kevin Berry

Hometown: Collinsville, Illinois

Major: Biology

University: Grinnell College

Faculty Mentor: Dr. Joel Maruniak, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

The effect of lipopolysaccharide (LPS) on neurogenesis in the nasal epithelium of mice

Kevin Berry and Joel Maruniak

Lipopolysaccharide (LPS) is a compound commonly found on the outer membrane of most Gram-negative bacteria and has been found to cause fever, lethargy, changes in sleep patterns, as well as many other symptoms typically related to infections. Many previous studies have shown that common stressors and depression can decrease the rate of neurogenesis. The obviously stressful effects of LPS make it ideal for a neurogenesis study. In this experiment, we wanted to determine if LPS treatment decreased the rate of neurogenesis in the olfactory epithelium. Mice were injected with 1 mg of LPS or given a sham injection for 2 successive days. On the third day, the mice were killed and the nasal epithelium was fixed with Bouin's solution. The nasal epithelia were then embedded in paraffin and sectioned. The sections were then processed for immunohistochemistry using a monoclonal antibody to PCNA. PCNA is a cell cycle protein and is a commonly used marker for mitosis. By counting PCNA-labelled cells we were able to compare the relative rates of neurogenesis in the olfactory epithelium of LPS and control mice. Blind counts of the labeled cells were made. A comparison of the average number of newly produced neurons between the control group and the group that had been injected showed that mice in the control group had an average neurogenesis rate that more than doubled that of the LPS treated animals.

John Daniel Bisges

Hometown: Jefferson City, Missouri

Major: Biology

University: Truman State University

Faculty Mentor: Dr. Andrew McClellan, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Fluorescent labeling of the neurons in the trigeminal nerve system of larval lamprey

John D. Bisges and Andrew D. McClellan

In vertebrates, many parts of the central nervous system are organized topologically. For example, sensations from different parts of the body are transmitted to or mapped to specific parts of the sensory cortex, where the sensations are perceived. In addition, the motor cortex is organized topologically, such that certain areas control movements of the legs while other areas control the upper parts of the body. In vertebrates, the trigeminal system includes sensory and motor neurons that project in the trigeminal nerve and are responsible for sensation and movements, respectively, of the head and oral hood (anterior head area). Sensory neurons receive inputs from the oral hood and project their axons into the brain in the descending trigeminal tracts (dV), where they make synaptic connections with other neurons. Motor neurons are located in the brain in the trigeminal motor nucleus (nVm) and project their axons to the periphery to innervate muscles in the head and oral hood. In the lamprey, a lower vertebrate, the trigeminal system is similar to that in other vertebrates. The purpose of the present study was to determine if the trigeminal system is topologically organized in normal lamprey, and to determine if this organization is restored following injury of the trigeminal nerve and axonal regeneration. First, two different fluorescent tracers (TRDA and FDA) were applied to different areas of the oral hood to determine if dV and nVm are topologically organized. Specifically, TRDA was applied to the medial areas of the oral hood so that it would be taken up by sensory and motor axons in the trigeminal systems and transported to the brain; FDA was applied to the lateral areas of the oral hood. If the trigeminal system in the lamprey is organized topologically, it is expected that medial regions of the dV and nVm would be labeled with TRDA while medial regions would be labeled with FDA. Where results were obtained, this was the case. Second, the trigeminal nerve was crushed on one side of the brain with fine forceps. Following a 4-week recovery period, axons of sensory and motor trigeminal neurons have regenerated, and there is recovery of sensory and motor function of the trigeminal system on the lesioned side. At this time, the two fluorescent tracers were again applied to medial and lateral parts of the oral hood. Here results were less concrete, as less neurons were present than in those animals that had not been injured, making labeling and viewing difficult. Further testing will be needed to better determine if damaged neurons regrow randomly or if they synapse to the same locations, which would indicate the presence of some sort of directing mechanism within the lamprey.

Malinda R. Boyd

Hometown: Blue Springs, Missouri

Major: Biological Engineering

University: University of Missouri-Columbia

Faculty Mentor: Dr. Andrew McClellan, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Variations in the parameters of locomotor activity with cycle time for larval lamprey

Malinda R. Boyd and Andrew D. McClellan

In normal larval lamprey and spinal cord-transected animals, locomotor activity in whole animals and *in vitro* brain/spinal cord preparations were studied to test whether two locomotor parameters, burst proportion (BP) and intersegmental phase lag, are constant with changes in cycle time (T). In individual animals, the slopes of BP and phase lag versus T were analyzed using linear regression. For all animals in a given group, the slopes for a given parameter were analyzed statistically to determine whether the locomotor parameters were relatively constant with changes in cycle time (T).

For locomotor muscle activity in normal whole animals, BP's increased with decreasing cycle times, while the phase lags were relatively constant. In contrast, in *in vitro* preparations from normal animals, the BP's and phase lags of locomotor activity both were relatively constant with changes in cycle time. In whole animals and *in vitro* preparations from spinal cord-transected animals, there was a strong tendency for locomotor parameters to vary with cycle time in a similar way as in normal animals.

For burst proportions, the differences in the slopes versus cycle time in whole animals and *in vitro* preparations are probably due to sensory feedback. For example, in whole animals, an increase in swimming speed and viscous resistance of water probably activates sensory feedback that increases the relative duration of muscle burst activity and the force of muscle contractions. Such sensory feedback would be absent in *in vitro* preparations.

In the lamprey, rostrocaudal phase lags are due to "short-distance coupling" between spinal oscillators in which coupling in the descending direction dominates and is much stronger than in the ascending direction (Hagevik and McClellan, 1994; McClellan, 1996). This short-distance coupling appears to be the main mechanism for producing constant rostrocaudal phase lags (McClellan and Hagevik, 1999; Hagevik and McClellan, 1999). Relatively constant BP's and phase lags ensure that the relative timing of locomotor burst activity is constant so that the basic S-shaped body form, which is the most efficient means of undulatory swimming, is retained at all swimming speeds.

Amy Leann Chamberlain

Hometown: Elsberry, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Dale G. Blevins, Agronomy

Funded by Life Sciences Undergraduate Research Opportunity Program

Developing an aeroponics system for study of rapid calcium and magnesium uptake by boron deficient roots

Amy Chamberlain and Dale G. Blevins

Boron is one of the seventeen essential elements needed for healthy growth and development of plants, however not much is known about its functions. When a plant becomes boron deficient the levels of other elements such as magnesium and calcium are altered. Changes in the boron deficient plant cause it to stop growing.

Boron deficiency can be a major problem for agriculture and forestry, therefore it is important to understand its mode of action and how it affects other elements in the plant. I have built an aeroponics system for growing boron deficient and boron sufficient squash plants. Atomic absorption was used to analyze and compare magnesium and calcium levels in the one centimeter root tip segments. Also potassium levels were measured by flame emmission.

The results, to date, show that magnesium levels drop in the boron deficient plants. However, calcium levels were higher in the minus boron plant. Calcium may be getting trapped inside the plasma membrane when boron is removed. This may be a result of inactive calcium efflux pumps. More experiments will be done to determine if calcium is really getting trapped. Also we will determine how quickly boron deficiency occurs and how long it takes to change the levels of magnesium and calcium in the root tips.

Summer Chaudhari

Hometown: Cape Girardeau, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Anand Chandrasekhar, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Roles of components of hedgehog signaling pathway in branchiomotor neuron induction in zebrafish

Summer Chaudhari, Gary Vanderlaan, Stephanie Bingham, and Anand Chandrasekhar

Branchiomotor neurons (BMNs) are located in the vertebrate hindbrain, and are responsible for functions such as gill and jaw movements in fish and facial expression, chewing and swallowing in humans. Hedgehog genes encode secreted proteins that induce many cell types during development, including motor neurons. In zebrafish, there are three hedgehog genes, *sonic hedgehog (shh),tiggy-winkle hedgehog (twhh)* and *echidna hedgehog (ehh)* . *Sonic-you* mutants are defective in *sonic hedgehog* and display a characteristic decrease but not complete loss of BMNs. Futhermore, all motor neurons are lost when both *shh* and *twhh* are removed through mutation and antisense morpholino injections. These experiments show that Shh and Twhh act cooperatively to induce BMNs. Mutations in the zebrafish *detour gene* , which encodes a Shh signaling pathway transcription factor *gli1* , lead to the complete loss of BMNs but not spinal motor neurons (SMNs). Development of other neurons and the induction of other Shh-mediated events occur normally in the mutant hindbrain, suggesting that *gli1* is specifically required for Shh-mediated induction of BMNs. Given the loss of BMNs but not SMNs in *gli1* mutants, it is possible that *gli1* and *gli2* have separate and common functions in BMN and SMN induction. We are addressing this issue by analyzing motor neuron development in *you-too* mutants, which are defective in *gli2* . Our preliminary analysis indicates that *you-too* function may also be necessary for inducing subsets of BMNs. These and other ongoing experiments will help clarify the roles of various hedgehog pathway components in inducing subsets of motor neurons in the vertebrate embryo.

Richard N. Clemon

Hometown: St. Louis, Missouri

Major: Electrical Engineering

University: University of Missouri-Rolla

Faculty Mentor: Dr. Mariesa Crow, Electrical Engineering, UMR

Funded by National Science Foundation - Access to Doctoral Education

Prony's Method of Analysis of Power System Data

Mariesa Crow and Richard Clemon

Prony's Method of Analysis is based upon the theory that any waveform can be modelled to fit a linear combination of sinusoidally decaying waveforms of the form A*exp(sigma*t)*cos(2*pi*f*t+phi). This project will use computer algorithms and program code in order to estimate the parameters of power system voltage data. The parameters of the data are the variables A, sigma, f, and phi over a specified time t. This will be accomplished by observing and analyzing the data and parameters of first, a small known function with and without noise, then of a large scale known function with and without noise. The information gained from the previous tests will be applied to a power system with limited known parameters in order to extract all parameters of all modes of data. This information will be used in order to develop algorithms which will sufficiently estimate an unknown power voltage waveform. The ultimate objective of the project is to extract sufficient data parameters in order to control power systems which will reduce pertubations in power networks.

Israel Aiesha Collier

Hometown: St. Louis, Missouri

Major: Biology

University: University of Missouri-St. Louis

Faculty Mentor: Dr. William Folk, Biochemistry

Funded by Summer Undergraduate Breast Cancer Research Program

Sequence elements important for expression of urokinase plasminogen activator (uPA) in cancer cells

Israel Collier, Kim Lieber, Olga Kenzior, and William Folk

Expression of urokinase plasminogen activator (uPA) and its receptor (uPAR) are prognostic indicators for the clinical outcome of many cancers. uPA activates numerous matrix metalloproteinases (a cascade of enzymes) and in tandem promotes proteolysis of the extracellular matrix (ECM). It is active during physiologic tissue remodeling that occurs during embryogenesis, pregnancy, angiogenesis, as well as wound healing. uPA is also involved in signal transduction and cell signaling. Expression of uPA is activated in cancer cells that become invasive and, eventually metastatic.

As an investigator in this project I have helped to analyze the expression of uPA in prostate cancer cells. The requirements for uPA expression in these cells are not known, but can be measured with a firefly luciferase reporter gene linked to the uPA promoter/enhancer (firefly luciferase is much more sensitively assayed than uPA). UPA gene 5' flanking sequences extending to -2100 contain a TATA promoter (-30) with proximal SP1-like sites (-80) at an enhancer region (-2100) that include PEA3-AP1 sites (-1600 to -2100). Introduction of the promoter and proximal SP1 sites (-80) gives the same luciferase activity on the full 5' flanking sequences through -2100. Deletion of the proximal SP1 sites eliminates luciferase activity. Co-expression of the MAPK (mitogen activated protein kinase) MEKK-1 enhances luciferase activity between -86 and -2100 constructs, but not of the -30 constructs. In summary, it appears that the proximal SP1-like sequences are responsible for full basil activity, and respond to MEKK activation. The PEA3-AP1 sites may contribute to MEKK activation.

We introduce the reporter genes into PC-3 (metastatic, express uPA) and LNCaP (non-metastatic, do not express uPA), prostate cancer cells, and after 36 hours lyse them and measure the amount of luciferase that is produced. Furthermore, we co-express constitutively active members of the MAPK pathway, known to be elevated in cancer cells that become metastatic.

Expression of uPA depends on the cell line in which it is tested, via transient transfections. It appears uPA is expressed in PC-3 cells and not expressed in LNCaP cells. Future experimentation will be required to investigate why this is the case. These studies will provide information regarding what regions will allow for the introduction of inhibitors that modify the signal transduction pathways and transcription factors believed to be responsible for activating uPA expression. If such experiments display these elements are responsible for uPA expression, future researchers will try to use that knowledge to block uPA expression in tumors, including breast cancer.

Pamela Nicole Conerly

Hometown: Jayess, Mississippi

Major: Microbiology

University: Mississippi State University

Faculty Mentor: Dr. Chris Carson and Dr. Ed Coe, Agronomy

Funded by Plant Genomics Internship at MU

F2 mapping of mutant genes in Maize using SSR markers

Pamela Conerly, Chris Carson, and Ed Coe

The mutant mapping section of the Missouri Maize project serves an important genetic research function. Mutants are important because they identify genes and each mutant gene conditions a phenotype that reveals something about the function of the gene. Currently, we are mapping an extensive set of mutants with a broad set of phenotypes that affect all stages of the plant's development. We are interested in genetically identifying the locations of the mutant genes in the genome, understanding which genes are involved with the various developmental and physiological processes of the plant, and understanding the identity of the normal gene.

To map mutants we first need to produce F1 families by crossing mutant lines with up to four different inbred lines. Next, the F1 progeny are grown and self-pollinated to produce F2 individuals from which we select homozygotes for mapping. We map mutants with molecularly based SSR markers because they are easy to use, they are highly polymorphic, and because they have been previously mapped to identify which chromosome and where on the chromosome the mutant gene is located. If a gene is located on a different chromosome than the SSR marker analyzed, then they are not linked. If a gene is located on the same chromosome as the SSR marker they are linked. The closer a gene is to a particular marker there will be fewer crossovers or recombinations between them, which suggests linkage. A gene is not linked if there is 50% of crossovers. To identify which SSR markers are useful in mapping a particular mutant family, we use bulk-segregant analysis by first comparing two classes of mixed samples: The first pool contains a mixture of DNA samples from plants that are 1/3 homozygous and 2/3 heterozygous for the mutant allele. The second pool contains DNA samples from plants that are all homozygous for the mutant allele. If the band pattern is different then the SSR is linked to the mutant gene. Using polymorphic SSR that show linkage in the BSA-screen the map position of the mutant gene is quantitated. We characterize the band pattern for each individual homozygote to determine two-point and three-point map data. The two and three-point map data determines the gene's specific map position on that chromosome.

In the lab, several BSA screens were run on a variety of mutants; *cfr*-2018* , *sr1* , *spc2* , *pg16* , *w*-8345* , *w*-4791* , and *al*-2003-2* . Presently, I am working to map the *spc2* mutant gene. We performed two different experiments: The BSA screen which led to a qualitative analysis of the data and the individual homozygous run which will yield the quantitative results of the experiment.

The results from the experiments performed this summer will provide more information for researchers to utilize in the completion of mapping the maize genome. In conjunction with future experiments, we hope to provide the bin locations of many of the unplaced mutants and precisely determine map positions of previously placed mutants.

Jeremy Cravens

Hometown: Fulton, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Wayne McDaniel, Cardiothoracic Surgery

Funded by Life Sciences Undergraduate Research Opportunity Program

Transthoracic defibrillation with multiphasic truncated exponential waveforms

Jeremy Cravens and Wayne C. McDaniel

In the United States, the leading cause of death in the population as a whole is cardiovascular disease. A little over 12 million (~20%) of the people with cardiovascular disease can be described as having coronary heart disease, the condition that most commonly leads to sudden heart attack (American Heart Association). Immediate or rapid electrical defibrillation has been shown to significantly improve chances of surviving a heart attack. Small, automated external defibrillators (AEDs) are now being mounted in a variety of public places, as well as in ambulances. These devices deliver an electrical shock to the patient according to a preset waveform. A waveform is a varying pattern of voltage over a very short period of time.

There have been many of these waveforms developed with ever-increasing efficacies. Due to the frequency content theory, it has been shown that waveform efficacy increases as more energy is delivered near 100 Hz. Waveforms with higher numbers of phases seem to deliver more energy in the 100 Hz range. Our goal is to verify this theory by testing waveforms of two, three, four, and five phases.

In our study, we have compared the defibrillation efficacy of four classes of waveform (biphasic, triphasic, quadriphasic, and pentaphasic) at an impedance level of 85 ohms, chosen to simulate average human transthoracic impedance. The four waveforms were delivered to fibrillating canines (n=6, 16.9±.9 kg) in random order twice to estimate an ED50 (50% dose). Voltage in each subsequent delivery of one waveform was incrementally decreased or increased depending on success or failure respectively.

The results of this study were pending when this abstract was due, but they should be ready for the poster session. With our results we hope to answer the question of whether or not increasing the number of phases in the waveform will increase the efficacy of the defibrillating shock or whether there is a place where increasing efficacy stops. Another question raised during the study was whether or not defibrillating shock is appreciably damaging the myocardial tissue. This topic would have to be addressed in another study.

Scott Culbertson

Hometown: Blue Springs, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jane M. Armer, Nursing

Funded by Summer Undergraduate Breast Cancer Research Program

An Overview of Post-Breast Cancer Treatment Lymphedema

Scott Culbertson, Jane M. Armer, and Julie Dusold

Current advances in the screening for and treatment of breast cancer are allowing more women to join a broadening segment of the population known as breast cancer survivors. The two million breast cancer survivors currently living in the United States now face a new problem, the condition known as lymphedema. Lymphedema is characterized by significant and persistent swelling associated with an abnormal accumulation of protein-rich fluid in the affected area (the arm on the side of the body affected with cancer), and literature suggests that between twenty and forty percent of breast cancer survivors will develop lymphedema at some time during their lives. The primary aim of this project is to assess the reliability, validity, sensitivity, and practicality of two physiological measures of limb fluid volume difference (sequential circumferential limb measurement and infrared laser measurement with the Perometer 400T) compared to water-displacement as the gold standard measurement. The secondary aim of this project is to examine the frequency of symptoms (i.e. tenderness, numbness, increased temperature, etc.), the actions taken to counteract these symptoms (i.e. manual lymph drainage, wrapping and bandaging of the arm, compression garments, etc.), and the psychosocial impacts of lymphedema (i.e. lifestyle changes, strain on family, etc.). The data are currently being collected and conclusive statements about both the primary and secondary aims of the current project cannot be made, but preliminary results are encouraging. This data will be essential in providing a working database for the upcoming five-year study of breast cancer patients, and their susceptibility to the development of lymphedema.

Cathey Cupples

Hometown: St. Louis, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Kathleen Newton, Biological Sciences

Funded by Plant Genomics Internship at MU

Analysis of inhibitor-induced mitochondrial stress responses in tobacco cells

Cathey Cupples, Shunxing Jiao, and Kathleen Newton

The NCS2 and NCS6 mutations found in maize mitochondria have adverse phenotypic effects due to their disruption of the elctron transport chain. NCS2 is a partial deletion of the *nad* 4 gene, which encodes a component of complex I (CI) and is characterized by pale-green leaf striping. NCS6 is a partial deletion of the *cox* 2 gene, which encodes a component of complex IV (CIV) and is characterized by narrow, yellow striped leaf sectors. Viable NCS plants are usually heteroplasmic due to the fact that homoplasmy for NCS mutations is lethal at certain stages of development because they occur in essential mitochondrial genes. The presence of these deletions in crucial mitochondrial genes leads to the induction of nuclear-encoded stress proteins, most notably, alternative oxidase, AOX, and a "heat stress" protein, HSP22. The specific stress-signaling pathway is yet to be determined, however it is known that both AOX and HSP22 levels increase considerably. The major purpose of this study was to determine whether the presence of inhibitors affecting CI and CIV induced the same stress response as that of the NCS2 and NCS6 mutations. Rotenone (inhibits CI, like NCS2) and antimycinA (inhibits CIV, like NCS6), were introduced to tobacco suspension culture cells. Total RNA was isolated at 0, 0.5, 1, 2, 4, 8, 16, 24, 48, and 72 hours. The RNA was then subjected to electrophoresis and transferred to nylon membranes. The membranes were hybridized with labeled *aox* and *hsp* 22 probes to look for induction of the messenger RNAs for these two stress proteins.

Anjuli Dahiya

Hometown: Cape Girardeau, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Karen Bennett, Molecular Microbiology & Immunology

Funded by National Science Foundation - Access to Doctoral Education

Ascaris suum : The search for the *Glh-1* gene in the parasite

Anjuli Dahiya, Victoria Blaho, and Karen Bennett

Ascaris suum is a parasite, which is a common cause of intestinal disease in an estimated one-third of the world's population. This parasite has been found in the intestines and feces of swine all over the world, which is why there is an immediate need for a cure. Some possible causes of this parasitic infection include wastewater reuse and unsanitary living conditions.

To understand the biology of *Ascaris suum* , we have chosen to analyze the simpler *Caenorhabiditis elegans* , which is a free-living nematode (roundworm). The *C.elegans* genome contains four germ line helicases (GLHs) and studies have shown that manipulation with *glh-1* and *glh-4* results in 100% sterility. Related experiments in the laboratory show that *Ascaris suum* contains a gene comparable to the *glh-1* in *C.elegans* , so a focus of the Bennett laboratory lies on using various Polymerase Chain Reaction (PCR) and RNA interference (RNAi) techniques to isolate and test the *Ascaris glh* genes. The first test after isolation will be to use RNAi injections to see if we can produce sterile *C.elegans* progeny. If this strategy is successful, then we will attempt a new RNAi feeding method in pigs infected with *Ascaris* , which could eventually lead to a novel means to control this disease.

Brittainy Dark

Hometown: Birmingham, Alabama

Major: Biology

University: Florida A&M University

Faculty Mentor: Dr. Gary A. Weisman, Biochemistry

Funded by National Science Foundation - REU (Life Sciences)

Probing the functional significance of the C-terminal domain of the $P2Y_2$ nucleotide receptor

Brittainy Dark, Sriparna Bagchi, Gary A. Weisman, and Laurie Erb

The human $P2Y_2$ nucleotide receptor for extracellular ATP and UTP is a G-protein coupled receptor whose activation leads to the phosphorylation of intracellular signaling molecules including the MAP kinases, ERK1 and ERK2, which are proteins that regulate cell proliferation and differentiation. The C-terminal domain of the $P2Y_2$ receptor contains a proline-rich region that acts as a Src homology-3 (SH3) binding domain to directly link the receptor to the MAP kinase cascade. The presence of serine and threonine residues, which are consensus phosphorylation sites for MAP kinases within the SH3 domain, suggests that upon their phosphorylation, the C-terminal domain of the $P2Y_2$ receptor acts as a WW binding domain known to promote the ubiquitination of proteins. Preliminary data indicates that the immunoprecipitated $P2Y_2$ receptor is ubiquitinated in a UTP-and time-dependent manner. A mutant receptor has been prepared in which the serine and threonine residues in the SH3 domain of the $P2Y_2$ receptor have been replaced by alanines. This mutant receptor is currently being expressed in a $P2Y_2$ receptor-null cell line to determine whether the absence of phosphorylation sites for MAP kinases prevents the ubiquitination of the receptor on an upstream lysine residue. Future studies will determine the functional relevance of the loss of the WW domain from the $P2Y_2$ receptor. It is postulated that the deletion of this domain will produce a receptor that is resistant to agonist-induced desensitization and/or receptor downregulation. (Supported by grants from the NIH, the American Diabetes Association, and Food for the 21st Century Program.)

Kelli N. Dixon

Hometown: Pine Bluff, Arkansas

Major: Biology

University: University of Arkansas-Pine Bluff

Faculty Mentor: Dr. Jan A. Miernyk, Biochemistry

Funded by National Science Foundation - REU (Life Sciences)

Construction of a plasmid for bacterial expression of the plant J-domain chaperone protein atDjC6

Kelli N. Dixon, Adam D. McDowell, Yuying Suo, and Jan A. Meirnyk

The *Arabidopsis thaliana* genome includes an unexpectedly large and diverse family of J-domain chaperone proteins, many of which have no counterpart in animal or microbial cells. While it can be assumed that these J-domain proteins interact with the 70 kDa stress proteins to form a chaperone complex that participates in protein folding, identifying specific target proteins of this complex and defining specific roles played by the J-domain proteins are a daunting problem. This problem is exacerbated by the small size of the plants being studied in the low abundance of the proteins of interest. A plasmid was constructed that directs expression of atDjC6 by *Escherichia coli* , in order to obtain sufficient protein for biochemical characterization. The plasmid encodes the plant chaperone protein fused to a bacterial maltose binding protein, which will serve as an aid to subsequent purification. A specific protease site was included between the maltose binding protein and atDjC6 in case it becomes necessary to remove the purification aid.

Ryan L. Dodson

Hometown: Scott City, Missouri

Major: Art History

University: University of Missouri-Columbia

Faculty Mentor: Dr. Steve Morris, MU Research Reactor

Funded by National Science Foundation - REU (MU Research Reactor)

Measurement of Selenium Status in a Canine Prostate Cancer Model

Ryan Dodson, Amanda Williams, Vickie Spate and Steve Morris (University of Missouri-Columbia, Research Reactor Center, Columbia, MO), and David Waters and Carol Oteham (Purdue University, School of Veterinary Medicine, Comparative Oncology Program, West Lafayette, IN)

Elderly intact male dogs have been found to be a useful prostate cancer (PC) model relative to the human. Supplemental dietary selenium and dehydroepiandrosterone (DHEA) are proposed chemopreventive agents against (PC). The objective of this study was to investigate the distribution of selenium in prostate (P), brain (B), heart (H), skeletal muscle (M) and liver (L) from dogs randomized to either a control group or one of 5 treatment groups. Toenails (N) were also analyzed as a biologic monitor. Selenium was quantified by instrumental neutron activation analysis at the MU Research Reactor by inducing the radioactive Se-77m (half life = 17.4 seconds) state and measuring the gamma-ray at 162 keV associated with its decay. Thirty nine (39) male dogs, 35 elderly and 4 young, 34 intact and 5 castrated, were randomized to the following groups: elderly castrated control (ECC, n=5), elderly intact controls (EIC, n=5), young intact controls (YIC, n=4), elderly low-dose selenomethionine (ELSeM, 3 mcg/kg/day, n=5), elderly high-dose selenomethionine (EHSeM, 6 mcg/kg/day, n=5), elderly low-dose selenium yeast (ELSeY, 3 mcg/kg/day, n=5), elderly high-dose selenium yeast (EHSeY, 6mcg/kg,day, n=5) and elderly DHEA (EDHEA, ~100mg/kg/day, n=5). The elderly dogs were maintained in their control and treatment groups for approximately 220 days at which time they were sacrificed and P, B, H, M and L tissue samples were collected. For each elderly dog, N specimens were collected at the beginning, middle and on the sacrifice date of the trial. Pearson correlation coefficients were computed for selenium concentrations between each pair of tissues and with the third N collection. In support of its routine use as a measure of selenium status in the human, the selenium concentration in the N specimen was positively (and statistically significantly $p < 0.0001$) correlated with all of the other tissues. Interestingly, the Se concentration in the ECC group is significantly lower in P ($p=0.003$) and B ($p=0.004$), but not in N ($p=0.43$), H ($p=0.19$), M ($p=0.20$) or L ($p=0.64$) compared to the EIC group. Consistent with the observed decline in Se status with age, we found the Se concentration in N to be lower in the EIC group compared to the YIC group ($p=0.006$). Selenium supplementation increases Se status in all tissues for both the SeM and SeY supplement types proportional to the dose. Se concentrations in P and L of animals from the EDHEA group are higher than corresponding controls and this difference approaches statistical significance ($p=0.13$ and 0.11, respectively). Distribution of Se by treatment and tissue will be presented.

Timothy Dryer

Hometown: Kansas City, Missouri

Major: Mathematics

University: Longview Community College

Faculty Mentor: Dr. John Urani, Physics, UMKC

Funded by National Science Foundation - Access to Doctoral Education

Examination of special relativity and the Lorentz-Dirac equation

Timothy Dryer and John Urani

The Lorentz-Dirac Equation has a century-old history, having fascinated theoretical physicist such as Lorentz , Schott, Dirac, and Rohrlich. Recent numerical solutions to the equation with realistic electron parameters have shown that many of features of quantum mechanical Compton Scattering can be reproduced, such as momentum transfer and classical *brehm strahlung.* However, at high frequencies incident electromagnetic waves do not cause electron scattering with large transverse momenta. A possible augmentation of the Lorentz-Dirac equation (a general solution) may produce more realistic results .* The proposed generalization of the equation is to include dipole structure for the charged particle , i.e. adding interaction terms which conatain a relativistic, covariant interaction between a dipole tensor and the electromagnetic field tensor. Preliminary work has shown that the basic interaction terms are easily constructed, however radiation-reaction terms and Schott-like terms (for mathematical consistency) are not trivial constructions. After the appropriate Lagrangian is constructed, extant computer algorithms can then be employed to integrate the twenty-five dimensional, coupled, non-linear, non-homogenous set of ordinary differential equations governing the system. It is expected that the system will exhibit chaotic behavior which has already been seen for the simple case of the unstructured point particle.

Julie Dusold

Hometown: Milwaukee, Wisconsin

Major: Interdisciplinary Studies

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jane Armer , Nursing

Funded by Summer Undergraduate Breast Cancer Research Program

Effectiveness of laser perometry for measuring limb volume

Julie Dusold, Jane Armer, and Scott Culbertson

Lymphedema, specifically a persistent swelling of the arm, affects many women as a result of breast cancer treatment. Two million women with breast cancer in the United States are at risk for developing lymphedema subsequent to breast cancer treatment. Imprecise measurement of limb fluid volume is detrimental to the diagnosis and treatment of this affliction. Establishing a reliable protocol for assessing lymphedema in women with breast cancer will certainly improve prevention and treatment, and consequently improve the quality of their life.

The traditional water-displacement method for measuring limb volume in the laboratory is impractical in a clinical setting. Sequential circumferential limb measurement, commonly used clinically, is time-consuming and less accurate. Using infrared laser perometry to measure limb volume may prove to be more precise and more practical.

Forty women with post-breast cancer treatment lymphedema and forty healthy volunteers are currently experiencing a series of laboratory assessments comparing limb volume measurement methods. At two points in time with a two-hour interval on the same day, each subject is measured with sequential limb circumferences, infrared laser perometry, and water displacement. In this way, the stability of each modality over time can be compared, and the most reliable protocol for limb volume measurement will be determined.

Preliminary findings indicate that infrared laser perometry is an effective method for measuring limb volume. According to data obtained by measuring twenty-six women with lymphedema, laser perometer measurements were consistently about 180 milliliters larger than corresponding water displacement measurements. This discrepancy is most likely due to human error while measuring with the volumeter. In fact, these findings suggest that the perometer may measure more of an affected arm accurately than the water displacement method. Completion of this experiment may yield results that establish a new standard of care for diagnosing and monitoring lymphedema with the laser perometer.

Brett Emo

Hometown: Sedalia, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Shari Freyermuth and Dr. Joseph Polacco, Biochemistry

Funded by Life Sciences Undergraduate Research Opportunity Program

Soybean Eu3 involvement in Ni binding and urease activation

Brett Emo, Shari Freyermuth, and Joe Polacco

Many forms of life utilize urease in order to preserve and provide useable nitrogen allowing the synthesis of protein required for cell division and growth. Urease converts urea, produced by many organisms as a nitrogen waste product, into ammonium through hydrolysis. Soybean urea is a prime candidate for study since the soybean is widely planted across the globe and serves as a major source of nutrition especially in terms of protein. A long term goal would be to improve the effectiveness of the enzyme so that plants will grow quickly in low soil urea concentrations allowing a reduction in the amount of urea fertilizer that is applied by farmers.

This project is concerned with the binding of nickel (Ni) to an accessory protein of soybean urease coded by the Eu3 gene. This gene is believed to be responsible in Ni binding due to a histidine-rich region near the N-terminus. In turn, the protein is then believed to be responsible for Ni insertion and activation of apo-urease. To test the significance of the Eu3 gene in binding nickel, the 5' end of the gene encoding the histidine-rich amino terminus of the protein was removed. The mutant gene was then cloned into bacteria and the resulting protein is to be examined for any Ni binding property through the use of a Ni binding column. Furthermore, the mutant gene will be cloned into urease negative soybean callus and checked for complementation.

This is a work in progress that will continue through the fall.

Dirk Erickson

Hometown: Blue Springs, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Abraham Eisenstark, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Variability in generation times and cold-shock response in archival cultures of *Salmonella typhimurium*

Dirk Erickson, Kelly Edwards, and Abraham Eisenstark

Decades ago, over 10,000 cultures of *Salmonella typhimurium* were sealed in agar stab vials for the purpose of mapping the bacterial chromosome. Our laboratory currently maintains this unique collection of isolates, stored at room temperature in agar stab vials. Even after more than 40 years of storage in an environment of limited nutrition, cells can be recovered from undried vials. Recovered mutants usually form 10^3-10^5 colonies per vial. These archival strains often differ from their original parental strains due to mutations accumulated during storage. These mutations most commonly include differences among amino acid requirements, sizes of the genome, and protein content.

This project focuses on 3 characteristics of cellular viability: 1) growth rate, 2) death rate, and 3) cold tolerance. Three carefully selected mutants followed the same life-cycle pattern compared to a wildtype control; i.e. slow initial growth followed by an accelerated death especially after cold shock treatment. Assays measuring basal metabolic functions can be used as an initial screening method to identify strains that have undergone genomic changes in response to environmental stresses in anticipation of more detailed molecular analysis.

Krista D. Fohey

Hometown: Wentzville, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Lene' Holland, Physiology

Funded by Life Sciences Undergraduate Research Opportunity Program

Do the neighboring DNA sequences of a known glucocorticoid responsive region play a role in hormonal induction of transcription?

Krista D. Fohey and Lene' J. Holland

Proteins are a critical factor in the regulation of gene expression. Specific proteins bind to particular areas of the DNA and interact with other proteins to turn on gene transcription. In Dr. Holland's laboratory, we are interested in how steroid hormones, primarily adrenal steroids, interact with their receptor proteins to regulate transcription. The specific gene for study of this process is the gene that codes for fibrinogen. Fibrinogen, the precursor to fibrin, is an important protein for blood clotting. Fibrinogen is made up of three subunits: A α, B β, and γ. Glucocorticoids, which are steroid hormones, regulate the synthesis of fibrinogen. In the frog *Xenopus laevis* , a liver nuclear protein called *Xenopus* glucocorticoid receptor accessory factor (XGRAF) was found to bind to a specific sequence of DNA in the regulatory region of transcription for the γ fibrinogen gene. XGRAF was also found to participate in a novel mechanism, heterodimerization with the glucocorticoid receptor (GR), to enhance activation of the gene by the glucocorticoid hormone.

To understand how XGRAF enhances GR function, the full extent of the DNA sequences necessary for hormone responsiveness need to be identified. The core binding sites for XGRAF and GR in the regulatory region of the γ fibrinogen subunit gene have been defined. In addition, the flanking sequences on both sides of the XGRAF and GR binding sites appear to be involved. However, the exact nucleotides that are important in the flanking sequences have not been identified.

We will determine whether the left or right flank is required for responding to the hormone using the transient transfection of primary heptatocytes system. Pieces of DNA containing the core sequences (XGRAF and GR) with the right and left flank sequences from γ fibrinogen gene were constructed. Similarly pieces of DNA containing the core sequences with the right and left flank sequences from B β fibrinogen gene were constructed. The pieces of DNA we want to study will be inserted into a DNA vector that also contains a reporter gene, the luciferase gene. To get the DNA into the cells electroporation is used. These cells are then put in a culture dish with or without glucocorticoid hormone and left to incubate for 48 hours. If our piece of DNA activates gene transcription, more light is produced from the luciferase gene. These experiments will distinguish whether the left and right flanking DNA sequences are important for XGRAF and GR to function together to stimulate transcription.

Derek Freund

Hometown: Gravois Mills, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. John Faaborg, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A comparison of average body condition of nestling Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*) as a determinant of habitat condition in evenaged and unevenaged forest treatments in Missouri Ozark Forest

Shelley Pasternak, Derek Freund, Salvador Luna-Garcia, and Garrett Rock

We conducted a pilot study in the Missouri Ozark Forest comparing the average body condition of bird species that nest in early succession habitats, Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*). We measured the nestlings when their feathers were just emerging from their shafts. Yellow-breasted Chats were 5-7 days old and Yellow-billed Cuckoos were 2-3 days old. We weighed and measured the tarsus and wing lengths of the birds. We then determined body condition using mass corrected for size. We compared the body condition of birds in evenaged and unevenaged treatments within each species. Yellow-billed Cuckoos showed no significant difference in body condition between the evenaged and unevenaged forest treatments. Likewise, Yellow-breasted Chats showed no significant difference in body condition between the evenaged and unevenaged forest treatments.

Darren Michael Gentry

Hometown: Savannah, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Judy Wall, Biochemistry

Funded by Life Sciences Undergraduate Research Opportunity Program

Methylation of *E.coli* plasmid DNA transformed into *Desulfovibrio desulfuricans* G20

Darren M. Gentry, Barbara J. Giles, and Judy D. Wall

The research project involved increasing the efficiency for transferring plasmid DNA from one bacterial genus, *Escherichia coli* , into another genus, *Desulfovibrio desulfuricans* G20, an anaerobic sulfate-reducing bacterium. *D. desulfuricans* has a defense mechanism, a type II restriction endonuclease, which cuts foreign DNA. An attempt was made to counter these type II restriction endonucleases of G20 by protecting the plasmid DNA from *E.coli* , pSC27, with methylating agents that methylated very frequently. Most type II restriction endonucleases do not recognize and cut the DNA when it is methylated. The foreign *E.coli* plasmid DNA, both methylated and unmethylated, was inserted into the electrocompetent *D. desulfuricans* by electroporation. The concentrations of the methylated plasmids were significantly less than that of the unmethylated that was electroporated into *D. desulfuricans* ; however, the number of transformants obtained from the electroporation were about equal. When corrected for concentration differences, the results show that the methylation of the foreign *E.coli* DNA does protect it from the *D. desulfuricans* defense mechanisms.

Karina A. Gilpin

Hometown: Acworth, Georgia

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Catherine Krull, Biological Sciences

Funded by National Science Foundation - Access to Doctoral Education

Roles for EphA4 and ephrin-A2 and ephrin-A5 in the patterning of muscle precursor cell migration

Karina Gilpin, Regina Hall, Rebecca McLennan, and Catherine Krull

The Eph family of receptors and their ligands consists of the largest known group of receptor tyrosine kinases. Eph family members control axon movements to their final destinations and may be important for proper muscle and limb development. Ephrin-A2 and -A5, ligands for Eph receptor kinases, inhibit axon growth invitro, suggesting that these factors act as repellents for migrating cells. We hypothesize that EphA4 and its ephrins-A2 and -A5, are needed for the normal migration of muscle precursor cells. To test this hypothesis, we have mice that are both wildtype and mutant for EphA4 and for ephrin-A2 and -A5. The goal is to compare muscle development in mice lacking these molecules versus wildtype mice. The results of our proposed studies will give us the opportunity to assess whether EphA4 and ephrin-A2 and -A5 lead to an abnormal navigation of muscle precursor cells in mouse embryos. The objective for this experiment is to examine the normal distribution of EphA4, and ephrin-A2/A5 in wildtype mice during the process of muscle development. We will analyze potential defects in muscle precursor cell migration in mice mutant for EphA4 and ehprin-A2 and A5, using immunocytochemistry and confocal imaging.

Natalie Goodin

Hometown: Columbia, Missouri

Major: Nursing

University: University of Missouri-Columbia

Faculty Mentor: Dr. Rebecca Johnson, Nursing

Funded by Undergraduate Research Mentorship Program in Nursing

Animal assisted activity and anxiety among radiation therapy patients

Natalie Goodin, Rebecca A. Johnson, Richard Meadows, Kathy Sevedge, Jenny Haubner, and Richard Madsen

Cancer is one of the most frightening and involved diagnoses that a patient can face. Not only must they confront the fear of death, but their long-term quality of life is also seriously threatened. This may result in anxiety and depression.

The variety of therapeutic and life-enhancing benefits of animal assisted activities has been documented with various populations but have been understudied with cancer patients. The purpose of the study is to ascertain to what extent an animal activity affects mood, self perceived health, and sense of coherence among these patients. A second purpose is to describe the extent to which the activity sessions may be stressful to the visitor dogs.

A convenience sample is being recruited of cancer patients age 21 and over who are undergoing radiation therapy. A total of 120 patients are randomly assigned to the experimental dog group (n=40), the human visitor control group (n=40), or the silent reading group (n=40), and participate in one of these interventions three times weekly for 4 weeks. Mood, anxiety, sense of coherence, fatigue, and self-perceived health are assessed before patients receive any intervention and after the 4-week period of 12 total visits. Data analysis will be completed using multiple regression techniques and analysis of variance.

Visitor dogs are assessed for any untoward effects of the multiple visits. Behavioral assessments of the dogs are conducted after each session, as are heart rate, respiratory rate, blood pressure, and urinary cortisol levels.

Data collection is currently ongoing.

Leslie Grill

Hometown: St. Louis, Missouri

Major: Fisheries and Wildlife

University: University of Missouri-Columbia

Faculty Mentor: Dr. Rex Cocroft, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

The use of substrate-borne vibrations in mate-localization in the treehopper species *Umbonia Crassicornis*

Leslie Grill

Treehoppers are one type of many insect species that communicate using vibrations. It uses plant-borne vibration to communicate in maternal-care and also in mate-acquisition. There have been few studies on the details of this type of messaging in treehoppers, specifically on mate localization.

We are interested in understanding if the male thornbug can localize the source of vibration while excluding other cues in order to determine if the thornbug can sense directionality using the vibration alone. It has been proven that this insect's body responds mechanically different when the vibration is sent from different directions. This suggests that it may use these differences to determine the direction of the vibration source. To test this, we play back pre-recorded female "calls" to male treehoppers sitting on the main stem of a y-shaped branch; each call is sent from one of the two sides of the Y. We observe the insect's behavior to ascertain whether it can locate the vibration source. It is expected that the ability to do so is advantageous to males who may be competing with others to locate a female.

Regina L. Hall

Hometown: Kansas City, Kansas

Major: Biology

University: Xavier University of Louisiana

Faculty Mentor: Dr. Catherine Krull, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Skeletal development and limb innervation: Potential roles for EphA4

Regina Hall, Karina Gilpin, Rebecca McLennan, and Catherine Krull

The Eph family of receptor tyrosine kinases (RTKs) consisting of membrane-bound Eph receptors and their ligands, the ephrins, are important molecular cues that influence axon guidance in developing embryos. The Eph family comprises two subclasses, EphA and EphB. The two classes are subdivided based on ligand binding preferences of the RTKs and sequence homology. Previous studies in chicken embryos have shown that EphA4 RTK is expressed in the dorsoproximal limb mesoderm, which forms the shoulder girdle, and by growing motor neurons. These results suggest that EphA4 RTK functions in skeletal development and axon outgrowth. We examined skeletal development in whole wildtype mouse and chick embryos at several different developmental stages, using Alcian blue staining. In an effort to determine the normal distribution of EphA4, lacZ staining was performed on EphA4 mutant mice and neurofilament antibody was applied to examine the organization of axons. In addition, EphA4 antibody was used to determine protein distributions in wildtype mice. These results serve as a foundation for future studies of skeletal and neural development in mice lacking EphA4 and their associated ephrins.

Joe Dee Haney

Hometown: Yelm , Washington

Major: Mechanical Engineering

University: University of Missouri-Columbia

Faculty Mentor: Dr. Frank Feng and Dr. William H. Miller, Mechanical & Aerospace Engineering and Nuclear Engineering

Funded by National Science Foundation - REU (MU Research Reactor)

Nuclear powered micro systems

Joe Dee Haney, William H. Miller, and Frank Feng

MEMS, micro electromechanical systems, are machines made using microchip fabrication techniques. The average size of these machines ranges from one micron to one millimeter. Since charged particles emanating from radioactive decay (such as alpha particles) dissipate their energy on the order of this dimension, they may be candidates for powering MEMS. This work explores this possibility.

Three possible energy conversions present themselves: nuclear to mechanical, nuclear to thermal to mechanical, and nuclear to electrical to mechanical. The first, nuclear to mechanical conversion, is the emphasis of this research.

To address this problem, a method of determining the amount of energy emitted perpendicularly to a given thickness of a planar alpha source was determined. Second, a study of the dynamics of a MEMS micro gyro was completed. The micro gyro is a two-degrees-of-freedom system that is driven into oscillations by an electrostatic comb drive. Determining the amount of energy required to drive the gyro into oscillations gives an idea of the feasibility of using the available nuclear energy to accomplish a similar task. Studying the dynamics of the micro gyro also enables its development for tactical grade guidance systems.

Melissa Hansen

Hometown: Newport News, Virginia

Major: Animal Science

University: Clemson University

Faculty Mentor: Dr. Eric Antoniou, Animal Sciences

Funded by F.B Miller Undergraduate Research Program in Animal Sciences

Comparative map of bovine chromosome 5

Melissa Hansen, Zhilin Liu, and Dr. Eric Antoniou

Dystocia (calving difficulty) is caused mainly by calves that are too large for the cow's pelvic opening. Dystocia can cause the death of a calf and/or cow, extended recovery for the cow, and in effect delayed return to estrus and more open cows. The losses due to dystocia are cost the beef industry an estimated to be $850 million annually. Producers currently focus on producing calves with large yearling weights; and therefore are producing calves with large birth weights as well. It is desired to be able to breed for large yearling weight and low birth weight in order to reduce the number of losses due to dystocia. A high linkage correlation exists between the genes for birth weight and yearling weight. Genes that code only for birth weight need to be located in order to breed solely for low birth weight. Previous studies have determined that a possible QTL (quantitative trait loci) for birth weight is located on bovine chromosome 5 (BTA5). Comparative mapping is used to pinpoint genes on BTA5. The current comparative map between BTA5 and HSA12 is insufficient due to lack of markers, leading to many gaps.

Previously in the study, specific genes were chosen based on the location on HSA12 and their sequence was run through a database in search of a corresponding bovine sequence. Primers were then designed to amplify DNA. During the course of the study, markers have been added to the current map to identify more linkage and fill the gaps that are present. 25 markers were previously added to the comparative map and 11 more were to be introduced. These markers were then mapped using polymerase chain reaction (PCR) a RH-12 panel. RHMAP3.0 was used to define linkage between the markers, as well as calculate the order.

Linkage was found among the 35 of the 36 total markers with a LOD score 4.0. The linkage was expected because the markers are located on the same chromosome. The one not included in the linkage group possibly due to its location on the chromosome. Before adding the new 11 markers, 11 of the original 25 markers were mapped using "rhmaklik" above LOD score 3.0. When the new 11 markers were added, 19 were mapped above LOD score 3.0. Using "rhmaklik", the 11 new markers were run with the previously ordered markers, 16 were mapped above a LOD score 3.0 and 24 are mapped with a LOD score above 2.0. When certain markers are fixed in order, certain markers from the new 11 will be added. If the order of the fixed markers is changed, other markers from the new group of 11 will be added. This illustrates that gaps are still present in the map. Therefore more markers will be added to continually lessen the gap in the map, in hopes to eliminate all gaps in the map and allowing the QTL for birth weight to be pinpointed.

Stacey A. Harley

Hometown: Harwood, Maryland

Major: Animal Science

University: University of Maryland Eastern Shore

Faculty Mentor: Dr. Duane H. Keisler, Animal Sciences

Funded by National Science Foundation - Access to Doctoral Education

Correlation between blood and milk serumleptin in goats and their offspring

Stacey Harley, Niki Whitley, Preston Buff, Beth McFadin-Buff, and Duane Keisler

In livestock, individual animal fat mass is a major determinant of the production performance of that animal and the value of that animal (i.e. animals with low fat mass produce poorly and have low commercial value). Fat cells, or adipocytes, play more than just a passive role of storing energy; they also function as a critical endocrine organ within the animal by secreting a hormone known as leptin. As levels of fat increase, leptin levels within the animal also increase. This endocrine signal serves to inform the brain of the body's composition. Furthermore, leptin has been found to be involved in other metabolic processes such as energy expenditure and distribution, thermoregulation, and feed intake/satiation. Leptin has now been detected in a multitude of species and biological fluids, however no data currently exists in the species of our interest - the goat. In particular, we are interested in the peri-parturient goat and the relationship between leptin levels in the blood and milk of the goat as a function of that animal's fat mass (commonly referred to as body condition score) and the potential impact milk leptin levels may have on the offspring. Therefore, our objectives were: 1) to determine if goat milk serum contains leptin and to determine if there is a relationship between milk and blood serum leptin concentrations in postpartum does, and 2) to determine possible correlations between milk and blood serum leptin concentrations and body condition of does as well as to body weights of their subsequent offspring. Our hypothesis is that leptin will be present in goat milk and that a positive correlation will exist between body condition scores and serum leptin levels. Thus, 20-mixed parity Boer-crossbred (meat breed) does and their offspring were used. Approximately one week prior to kidding, does were assigned a body condition score (on a 1-5 scale), weighed and a blood sample collected via jugular venipuncture. Thus, blood and milk samples were collected relative to kidding (kidding = d0) on days -4, 0.5, 1, 3, 5, 7, 14, 21, 28, 35, 42, 49, and 56. Weights were also collected weekly on does and kids and body condition scores were assigned at this time to the does (days 21, 28, 35, 42, 49, and 56). Blood and milk serum leptin concentrations were determined using a double antibody radioimmunoassay now validated for use in goat blood serum and milk. Relationships between blood serum leptin, milk leptin, doe weight, kid weight, and doe body condition score were analyzed using a Pearson correlation coefficient. From our analysis, we found that as days postpartum increased levels of milk serum leptin decreased over time ($P = < 0.001$). In contrast, blood serum leptin increased to day 56 postpartum. A negative relationship existed between milk serum leptin concentrations and kid weight ($P = 0.0008$). As milk leptin decreased, kid weight increased. We conclude that leptin is present in the blood and milk serum of goats and the relationship between the two respond as independent depots.

Lauren Hart

Hometown: St. Louis, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Donald Riddle, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Are glucose levels a predictor of life span?

Lauren M. Hart, P.S. Albert, and D.L. Riddle

A gene encoding an insulin or insulin-like growth factor receptor in *C. elegans* mutants was originally identified and studied in the Riddle laboratory at MU. Mutations in this gene affect larval development and also increase (double) adult life span. This has spawned an extensive effort in the field of aging research to link insulin signaling function with the determination of human life span. The hypothesis being tested in this project is whether this receptor is a functional homolog of the insunlin receptor in humans.

A Glucose test kit designed for human blood serum is being adapted to assay both internal and external (excreted) glucose levels in normal worms [N2] and the long-lived insulin-like receptor mutant [*daf-2(e1370)*]. Thus far the absorbance readings used to determine the glucose levels are too low to draw conclusions. We are currently in the process of making adjustments to the protocol so that future experiments will yield more meaningful results.

Michelle A. Harwerth

Hometown: New Athens, Illinois

Major: Microbiology

University: Southern Illinois University, Carbondale

Faculty Mentor: Dr. Steven Van Doren, Biochemistry

Funded by Summer Undergraduate Breast Cancer Research Program

Thermostability of murine Polyomavirus J Domain mutants using circular dichroism

Ashley Anne Nenninger, Michelle A. Harwerth, Michael I. Riley, and Steven R. Van Doren

The polyomaviruses infect rodents and primates causing the formation of many kinds of tumors. They are widely used tools for studying signal transduction pathways important in cellular growth and transformation. During the onset of infection, the polyomavirus produces a set of proteins called the "tumor antigens" designated large, middle, small and tiny T antigens. A protein domain common to all four antigens, which is found at the N-terminus, is called the "J domain". It is a small, α-helical domain with a leucine hydrophobic core. The J domain is an important factor in expropriating host cell replication enzymes and disrupting the host cell's normal quiescent phase to cell division. This study analyzed mutants of the viral J domain whose phenotypes have been shown to be defective in transforming the host cell. The thermostability, characterized by the melting temperature (Tm), was measured in order to determine the contribution that various amino acid residues have in maintaining structure. We expected that residues mutated in the hydrophobic core (buried residues) to alter the thermostability more than surface exposed residues. Recombinant proteins were overexpressed in E. coli, purified, and analyzed by circular dichroism. The α-helical content was monitored at 222nm as temperature was increased. The average Tm for wild type J domain is 66.7 ^{o}C. Eight mutants were successfully evaluated; however, four mutations in the hydrophobic core could not be evaluated because no protein was acquired. These hydrophobic mutations greatly affected the protein's stability as expected. Other less severe mutations in the hydrophobic core moderately decreased the protein's stability, and one provided a slight increase. The Tm for the two loop mutations are not significantly different than WT. This suggests that these mutants' inability to replicate is not due to structural instability as previously thought. The Tm for three N-terminal mutations are slightly reduced by 5-9 ^{o}C, which is somewhat surprising considering the large surface exposure these mutants have. This instability may be part of the reason that these mutants are defective in transformation.

Courtney M. Hoshibata

Hometown: Bellevue, Washington

Major: Biology

University: Harvey Mudd College

Faculty Mentor: Dr. Arun K. Chatterjee, Plant Microbiology and Pathology

Funded by Plant Genomics Internship at MU

Occurrence and effects of a global regulator *rsmA* $_{DC3000}$ in *Pseudomonas*

Courtney M. Hoshibata, Hiroaki Hasegawa, Yaya Cui, and Asita Chatterjee

Pseudomonas syringae pv. tomato DC3000 is a host-specific pathogen that causes leaf spots in host plants tomato and *Arabidopsis* and necrotic hypersensitive response (HR) in nonhost tobacco. Screening of a DC3000 gene bank identified a functional homologue of *rsmA* $_{Ecc71}$, a negative regulator of pathogenicity factors in *Erwinia carotovora* subsp. *carotovora* strain 71 (Ecc71). The present study sought to investigate the occurrence and functionality of *rsmA* $_{DC3000}$ in other *Pseudomonas* species.

Southern hybridization and PCR analyses established the presence of *rsmA* $_{DC3000}$ homologues in several *P. syringae* pv. syringae strains, *P. viridiflava*, and *P. syringae* pv. phaseolicola, but not in *P. fluorescens* or *P. corrugata*. Many pigment producing strains carrying multiple copies of *rsmA* $_{DC3000}$ exhibited reduced levels of pigmentation and protease production. Since *rsmA* $_{Ecc71}$ negatively regulates HrpN production in Ecc71, transcript assays may indicate reduced *hrpZ* transcript levels in *Pseudomonas* strains carrying multiple copies of *rsmA* $_{DC3000}$ compared to those of wild types.

These preliminary results support the finding that *rsmA* $_{DC3000}$ functions as a negative regulator in several *Pseudomonas* strains, though the precise mechanism of action remains to be determined. To further confirm the effects of *rsmA* $_{DC3000}$ research to obtain negative mutants is currently in progress.

Michael Hughes

Hometown: St. Charles, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jeffrey Anglen, Orthopaedic Surgery

Funded by Life Sciences Undergraduate Research Opportunity Program

Increased nonunion rate following use of indomethacin

Michael Hughes, Timothy Burd, and Jeffrey Anglen

Introduction: Heterotopic ossifcation (H.O.) of the hip joint is an often, debilitating complication following acetabular fractures. Previous research has indicated that single dose focal radiation or a 6 week regimen of the nonsteroidal anti-inflammatory drug (NSAID), Indomethacin, is equally effective as a H.O. prophylaxis. Indomethacin is presently the standard treatment because it is 200 times less expensive than radiation therapy. However, delayed bone maturation has been cited as a possible side effect of NSAID's. This study seeks to find out if acetabular fracture patients with concomitant long bone fractures are at higher risk for another serious complication, fracture nonunion, by taking indomethacin for H.O prophylaxis.

Methods: A retrospective review of 282 patients who underwent open reduction and internal fixation(ORIF) by a single surgeon from July 1992 to January 2001. Data was recorded under a total of 72 variables for the 113 patients sustaining a long bone fracture.

Results: Patients who received indomethacin had a 25.6% incidence of long bone nonunion while those receiving radiation and no therapy had a 11.9% and 6.3% rate, respectively. Patients in each group had similar age, smoking habits, open fracture rate, and ISS. The nonunion difference between the no therapy and radiation group was not significant ($p=.22$). The difference between the non-indocin cohort and indocin treated patients was significant ($p=.031$). The nonunion rate odds ratio of indocin to nonindocin is 3.05, with 95% confidence for the odds ratio being between 1.11 and 8.41.

Conclusions: Indomethacin patients had a 3 times greater rate of nonunion. This suggests indomethacin should be avoided, if possible, in acetabular fracture patients with concomitant long bone fractures. The use of the more expensive focal radiation therapy is justifiable for heterotopic ossification prophylaxis, to achieve optimal prognosis for the patient.

Kandis P. Ingram

Hometown: Valrico, Florida

Major: Animal Science

University: University of Missouri-Columbia

Faculty Mentor: Dr. James R. Turk, Veterinary Pathobiology

Funded by National Science Foundation - Access to Doctoral Education

Immunohistochemistry for amylin, perlecan, and insulin in cats with non-insulin dependent diabetes mellitus and insular amyloidosis

Kandis P. Ingram and James R. Turk

Amyloid fibrils destroy pancreatic β cells and contribute to impaired insulin response in diabetic cats. Islet amyloid polypeptide (amylin) is the primary constituent of amyloid fibrils and is naturally produced by β cells; however, excessive amylin production characterizes a condition known as amyloidosis.

The hypothesis of this retrospective study is that pancreatic amyloidosis and amylin affect the amount of insulin that is immunolocalized in feline pancreatic islets. The objectives are to quantify amylin, perlecan (a heparan sulfate proteoglycan or HSPG), and insulin by immunohistochemical techniques in formalin-fixed, paraffin-embedded samples of pancreas in cats with and without pancreatic amyloid.

Formalin fixed samples from all test and control cats were stained with Bennhold's Congo red staining technique and examined by polarization microscopy at standard light intensity and angle of filter rotation. Color print photomicrographs at 200X magnification were generated, digitized, and analyzed for "sum of bright objects" using Image Pro software.

Sections of pancreas from all test and control cats were stained by routine avidin-biotin peroxidase immunohistochemistry using antisera to amylin (clone R10/99 mouse anti-human IgG_1 amylin peptide antibody, Serotec Laboratories), insulin (clone Z006 mouse monoclonal anti-insulin IgG_1 antibody, Zymed Laboratories), and perlecan (clone 7B5 mouse monoclonal anti-perlecan IgG_1 antibody, Zymed Laboratories). Digital images were captured at 200X magnification and analyzed for "sum of dark objects" using Image Pro software.

Results thus far indicate positive staining in test and control groups for amyloid using Congo red, positive staining for insulin immunohistochemical staining in test and control groups, and negative staining for perlecan immunohistochemical staining in preliminary trials.

Julie Janes

Hometown: Hannibal, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Kevin Fritsche, Animal Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

The role of neutrophils in dietary n-3 PUFA-mediated reduction of host resistance to *Listeria*

Julie Janes, Kevin Fritsche, Robert Irons, Lisa Pompos, Meijuan Zhang, Rachel Claunch, and Andy O'Brien

Diets rich in omega-3 polyunsaturated fatty acids (n-3 PUFA), such as from fish oil, significantly reduces murine resistance to *Listeria monocytogenes* infection. This impact on resistance results in part, from a diet-induced decline in IL-12 biosynthesis, an important immune activating cytokine. During the early stages of Listeriosis, polymorphonuclear cells (PMN / neutrophils) play an important role by phagocytoseing bacteria and producing pro-inflammatory proteins, including IL-12. This experiment is designed to test our hypothesis that dietary n-3 PUFA-mediated reduction of *in vivo* IL-12 production and host resistance to *Listeria* is neutrophil dependent. To study the significance of neutrophils, we deplete these cells using an RB6-8C5 monoclonal antibody.

In this study, healthy 4-5 week old female Balb/c mice were fed one of three nutritionally complete experimental diets that varied only in their source of fat (i.e. lard, soybean, or menhaden fish oil). After 8 weeks, half of the mice in each diet were depleted of neutrophils (via 200 ug of RB6 mAB treatment) 24 hours prior to receiving an intraperitoneal injection of 10^5 c.f.u. of *Listeria* . A number of each diet group is represented by control mice and RB6 treated mice. By comparing the RB6(+) mice to the control mice we can determine the role neutrophils are playing in the reduced resistance to *Listeria* among the fish oil fed dietary group. This experiment has shown that the RB6 mAB treatment successfully reduced neutrophils in all diet groups, and an n-3 PUFA diet does not decrease the amount of neutrophils at the site of infection. Studies are currently being conducted to determine if depleting neutrophils affect the concentration of IL-12 detected in the blood, and if dietary n-3 PUFA alter the amount of IL-12 produced during a *Listeria monocytogenes* infection.

Matthew Wade John

Hometown: Blue Springs, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Andrew McClellan, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Mapping the locomotor command system in the lamprey brain

Matthew W. John and Andrew D. McClellan

One of the goals of neuroscience is to determine the locations of neurons and their interconnections in the nervous system that perform certain functions. For example, anatomical tracers, such as biocytin or horseradish peroxidase (HRP), injected into specific areas of the brain are taken up by axons and retrogradely transported to neuronal cell bodies, where it can later be reacted and visualized. Biocytin has a distinct advantage over HRP due to its smaller molecular size, which allows faster transport times and an increased ability to diffuse into the smallest areas of the neuron. In the lamprey, a lower vertebrate, we are interested in the locations and interconnectivity of brain neurons that are part of a command system which activates motor networks in the spinal cord to initiate locomotion. The lamprey has the advantage that its entire brain can be histologically processed as a "whole mount", which allows three dimensional visualization of labeled neurons in the CNS without the need for sectioning the tissue. Having a circuit diagram of the lamprey command system is important to understand how this system functions in normal animals, and perhaps more importantly, the pathways by which command neurons in the brain regenerate their axons following spinal cord transection. First, in the present study, HRP or biocytin were pressure ejected, using a glass micropipette, into different areas of the lamprey brain to determine the locations of neurons that might be important for the initiation of locomotion. For example, pressure ejection of these tracers into reticular nuclei, which contain reticulospinal (RS) neurons that project to the spinal cord and initiate locomotion, labeled some neurons in the lateral part of the rhombencephalon as well as some neurons in the mesencephalon. These neurons probably are presynaptic to RS neurons and may be important for the initiation of locomotion. Typically, biocytin had a shorter transport time and a greater ability to label dendritic and axonal projections not seen with HRP. We are now in the process of optimizing this technique. Second, biocytin has been applied to the lamprey spinal cord to retrogradely label descending brain neurons. We expect that this labeling will be more complete than that with HRP and will provide a more detailed description of the locations and morphology of descending brain neurons, some of which are involved in the activation of spinal motor networks and the initiation of locomotion.

Kathryn S. Konrad

Hometown: Saint Louis, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Rex Cocroft, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

How do plant properties effect the transmission of vibrational songs of the treehopper species *Umbonia crassicornis* ?

Kathryn S. Konrad, Holly J. Shngart, and Krystall Y. Tibbs

The treehopper, *Umbonia crassicornis* , attracts mates by means of vibrational communication. As the male treehoppers travel from plant to plant they send vibrations through the stem of their host plant. We are interested in knowing how the signal filtering is altered on the different plants as well as how signals are altered at different distances on the plant. The two plant species, Albizia julibrissin and Viburnum prunifolium were selected due to their different physical properties. Males were individually placed on each of the plants where they produced a series of mating signals. These vibrational signals were recorded by laser vibrometry. The laser was focused on a piece of reflective tape, which allows the laser to detect Doppler-shifts in the frequency of the reflected light. The preliminary analysis suggests that different plants are filtering the frequency content of the calls in different ways. The temporal aspect of the calls, such as call length, is not significantly affected. Our goal is to determine if aspects of the signals due to variation depend on location of the treehopper.

Dana S. Lambert

Hometown: Buffalo Grove, Illinois

Major: Fisheries & Wildlife

University: University of Missouri-Columbia

Faculty Mentor: Dr. Ray Semlitsch, Biological Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

The effects of predation risk and food availability on *Bufo americanus* tadpoles

Dana Lambert, Nathan Mills, and Ray Semlitsch

Organization of grouping behavior can be highly variable, ranging from aggregates with no social organization to shoals, which exhibit highly organized social interactions. In anurans, tadpoles often form aggregates around clumped resources, such as food or habitat. We wanted to investigate the possibility of more socially complex shoaling behavior. Through coordinated group behavior, shoaling provides participating individuals with benefits they could not obtain by themselves, such as increased safety from predation or increased foraging success. Changes in shoaling behavior have been detected in fish in response to predator pressure and food availability. However, shoaling has not been well documented in anuran tadpoles. Our hypothesis is that *Bufo* tadpoles will in fact show shoaling behavior, and will increase shoal size and/or cohesiveness in response to predation pressure and food availablity.

In order to assess shoaling behavior in *Bufo* tadpoles, we set up 12 pools assigned to a "predator" or "no-predator" treatment and a "low food" or a "high food" treatment in a randomized block design. "Predator" pools contained two dragonfly larvae. Tadpoles were fed according to assigned food level every 24 hours. Digital photographs were taken of each pool for three consecutive days. The experiment was repeated three times over three week's time. At this time, point plots representitive of the tadpoles' postions in each photo are being created with ArcView GIS 3.2. From these plots, average nearest neighbor distances (R) will be determined across each pool, where $R<1$ shows clustered distribution, $R=1$ shows random distribution, and $R>1$ shows dipersed distribution. This statistical analysis will provide a clear picture of whether *Bufo americanus* tadpoles show shoaling behavior and, if so, how it is modified by food availability and predation risk. If tadpoles do show shoaling behavior, it would demonstrate a higher level of sociality in tadpoles than previously assumed, opening a door to further exploration of this question.

Latevi Lawson

Hometown: Los Angeles, California

Major: Chemistry

University: Xavier University of Louisiana

Faculty Mentor: Dr. Nuran Ercal, Biochemistry, UMR

Funded by National Science Foundation - Access to Doctoral Education

Toxicity assay by cellular colonization

Nuran Ercal, Nukhet Aykin, and Latevi Lawson

Lead poisoning has been studied for many years. It is documented to cause problems in many biological systems, especially the central nervous system, the renal system, the henopoietic system, and the immune system depending on the age and dosage received by exposed persons. Children are prone to suffer harsher effects associated with lead toxicity than adults. Exposure during childhood effects memory and attention span. Fetal exposure to maternal blood lead levels in the range of 10-15mg/dL is associated with reductions in mental development, gestational age, and birth weight in infants (T,2). In children a lead level of 20mg/dL is associated with decreased IQ. Lead, however has not been shown to be directly responsible for the diseases and adverse effects which correlates to its presents. Lead is, however, responsible for the generation of reactive oxygen species (ROS), which will react with the cellular membrane, disabling proper membrane function, which causes the premature death of cells. It is this premature death of cell that may be responsible for disease instantiation.

Antioxidants defend against ROS by scavenging the unpaired valence electron that make the species reactive, preventing a ROS attack against the membrane. Glutathione (GSH) is a cellular produced biomolecule that serves as the body's primary defense against ROS. This Experiment has been designed to test the antioxidant capability of Selenium in conjunction with the bodies own radical scavenging system.

Twenty-seven petri dishes were seeded each containing 100 Chinese Hamster Ovary (CHO) cells. The cells were introduced to different concentrations of the selenium cysteine (SeCys) solution to determine safe concentrations of solution that would also yield the best results: The survival curve indicated that 5mM of SeCys solution was the highest usable concentration that would not terminate the CHO cells, we determined that 1mM would yield the best results. Four groups were seeded, with each flask containing a total volume of 28mL. The groups were comprised of a control, a lead (Pb) only group, a SeCys, and a Pb-SeCys. 280mL. of lead acetate (PbAc) was added to the Pb and SeCys groups, making their total PbAc concentration 500mM. The control and SeCys groups received 280mL of HPLC water as opposed to PbAc. The four groups were left over night and followed by a media change. The Pb and control groups received plain media, while the SeCys and Pb-SeCys received 1mL of solution from 2.5mM tube and added to a total volume of 25mL, the total concentration of SeCys in the flask was 1mM. The cells were trypshihized and centrifuged and pellets keep at -70mC and will remain until GSH, oxidized glutathione (GSSG), malondialdehyde (MDA), and catelase analyses. We are expecting selenium to add added protection against the ROS generated by Pb.

Kristen Leach

Hometown: Palestine, Texas

Major: Genetics

University: Texas A&M University

Faculty Mentor: Dr. Georgia Davis, Agronomy

Funded by Plant Genomics Internship at MU

Variations of the *c2* Gene in Maize and their Relationship to Aflatoxin Accumulation

K. Leach, D. Liu, J. Frost, T. Musket, and G. Davis

Aflatoxin contamination has caused corn producers problems for many years. A fungus known as, *Aspergullis flavus* , produces this carcinogenic toxin. This fungus also infects other crops, such as, cotton, peanuts, and fruits. The Food and Drug Administration puts strict regulations on the levels, 20 parts per billion, of toxin allowed to enter the market. When a shipment exceeds this level, it is sent back and destroyed.

Previous research has found a link between a gene that codes for chalcone synthase and the amount of toxin produced (Davis, unpublished). Three different alleles of the gene were analyzed relative to aflatoxin production: *C2, c2, c2Id-f* . When the crop possesses either *c2* (recessive allele) or *c2Id-f* (partially functional allele), there is a seven fold increase in the levels of toxin produced when compared to *C2* (fully functional allele). The objective of this project was to sequence the three different alleles, and compare the sequences to identify DNA changes associated with changes in toxin accumulation.

Through single nucleotide polymorphisms (SNPs) and yeast gap rescue methods, the three alleles were sequenced. Primers for these processes were picked from the gene sequence in GenBank using a primer selection program called Primer 3. A BLAST search was used to compare *c2* to white pollen (*whp1*) to select for primers that would specifically amplify *c2* , since *c2* and *whp1* have regions of homology. The primer analysis showed there is a deletion in *c2I-df* at 2387-2591 base pairs compared to the other two alleles. Larger amplification products were obtained for *c2I-df* with four of the primer pairs compared to *C2* and *c2* . This information will further the understanding in the *c2* sequence relative to aflatoxin accumulation levels. In addition, we hope to use this data to complete targeted cloning of the three alleles for use in in vitro complementation assays in *A. flavus pks* - (missing *c2* analog).

Danny Liu

Hometown: Springfield, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Georgia Davis, Agronomy

Funded by Life Sciences Undergraduate Research Opportunity Program

Associations Between changes in DNA Sequence of *Glossy15* and Juvenile Leaf Number

D. Liu, K. Leach, S. Szalma, J. Frost, T. Musket, E. Buckler, M. McMullen, and G. Davis.

There are two distinct leaf developmental stages in maize. One of the major physiological differences in the two stages is the leaf wax covering. The first leaves to develop are juvenile leaves and their leaf wax is a dull bluish-gray appearance. Fully adult leaves appear after a transitional stage and their wax has a glossy appearance. The number of juvenile and adult leaves varies among different lines of maize. Variation in number of juvenile leaves correlates closely to Lepidopteran resistance in the plant. Lines of maize with fewer juvenile leaves are more resistant to fall armyworm and southwestern corn borer infestation during the whorl stage. The difference in the adult wax coating is thought to be a principle contributor to this resistance.

The maize *Glossy15 (Gl15)* locus is a loss-of-function mutation that causes abbreviated expression of juvenile leaves. An experiment was conducted on 100 corn lines (50 U.S. inbreds, 20 European inbreds, 20 Tropical inbreds, and 10 relatives of maize) to identify the lines with the lowest and highest number of juvenile leaves. Seven primer pairs were selected from GenBank sequence U41466 for further evaluation, of the seven initial primer pairs, three amplified robust single fragments. These were selected for use in sequencing. A subset of DNA samples from thirty-two of the lines were sequenced using the three pre-selected single nucleotide polymorphism (SNP) primers to determine associations between changes in DNA sequence and in the number of juvenile or adult leaves. Our goal is to identify one or more SNP associated with reduced juvenile leave number that can be used to screen for more insect resistant corn lines.

Salvador Luna-Garcia

Hometown: Victoria, Tamaulipas, Mexico

Major: Biology

University: Instituto Tecnologico de Ciudad Victoria

Faculty Mentor: Dr. John Faaborg, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A comparison of average body condition of nestling Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*) as a determinant of habitat condition in evenaged and unevenaged forest treatments in Missouri Ozark Forest

Shelley Pasternak, Derek Freund, Salvador Luna-Garcia, and Garrett Rock

We conducted a pilot study in the Missouri Ozark Forest comparing the average body condition of bird species that nest in early succession habitats, Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*). We measured the nestlings when their feathers were just emerging from their shafts. Yellow-breasted Chats were 5-7 days old and Yellow-billed Cuckoos were 2-3 days old. We weighed and measured the tarsus and wing lengths of the birds. We then determined body condition using mass corrected for size. We compared the body condition of birds in evenaged and unevenaged treatments within each species. Yellow-billed Cuckoos showed no significant difference in body condition between the evenaged and unevenaged forest treatments. Likewise, Yellow-breasted Chats showed no significant difference in body condition between the evenaged and unevenaged forest treatments.

Logan Nichols

Hometown: St. Louis, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. John Faaborg and Dr. Paul Porneluzi, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Clear cut recapture rates over a period of multiple days

Logan Nichols

I am looking at the number of birds that we capture over a period of time in clear cuts. I am most interested in time periods of four days and two days. While I was netting birds I noticed that the numbers usually went down each day from the same location and I expect the trend to continue. The reason why recapture rates decline is because birds have learned to avoid the net. Along with studying the amount of recaptures, I also am looking at species captured and recaptured to give clues to why they are there. If we capture a large number of Red Vireos in the clear cuts it could be that they are using the area or migrating, but if we recapture them over a few days we know how long they are using the clear cuts. If the capture rate in general does not go down we know that new birds are continuously coming in to use the clear cuts. Where I capture them tells me what they could be doing and maybe how much time they spent it the clear-cuts. The forest birds might just soar through the middle or they might hang along the edge. It might be the same or different for the clear-cut birds. To find this or at least some clues to what happens I must first start with looking at the recapture and capture rates from the different locations. Preliminary data suggest that more birds tend to spend a lot of time along the edge of the clear cuts and less time in the center.

Darren R. Oakley

Hometown: Columbia, Missouri

Major: Fisheries And Wildlife

University: University of Missouri-Columbia

Faculty Mentor: Dr. John Faaborg and Dr. Paul Porneluzi, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Analysis of Avian Recapture Rates Between the Edge and Middle of Clearcuts

Darren R. Oakley

Regenerating clearcuts provide good habitat for a large number of birds that favor dense vegetation. The birds that require early succession growth typically confine their daily foraging activities to small territories. Forest breeding birds also move into the clearcut after their young leave the nest. These birds may not be territorial within the clearcut. I compared mistnetting data from three sites where birds were banded both around the perimeter and within the interior of the clearcut to provide information about the movements of these birds and the percentage that utilize a larger area. The clearcuts being studied have been regenerating for about five years. The mistnets are placed at points both around the edge and in the middle of the clearcut. Each point around the edge is 50 meters apart and points within the cut are 25 meters from the beginning of one net to the next. The Missouri Ozark Forest Ecosystem Project is a hundred year study that will provide the opportunity for biologists to understand the effects of different types of timber management upon the flora and fauna that reside in south-eastern Missouri. There are three control sites, three even-aged (clearcuts) sites, and three uneven aged (select cuts) sites. The evenaged sites consist of 60-80 year old forest with 10% being harvested every 10-15 years.

Renita C. Oko

Hometown: Grandview, Missouri

Major: Chemical Engineering

University: University of Missouri-Rolla

Faculty Mentor: Dr. Parthasakha Neogi, Chemical Engineering, UMR

Funded by National Science Foundation - Access to Doctoral Education

Equation of state using orthogonal polynomials

Renita C. Oko and Parthasakha Neogi

This study deals with the issue of knowing exactly the volumes of hydrocarbon products. These volumes are determined by factors such as pressure, temperature and compositions, and the equations used to calculate the volumes are based on these factors. In this study, I use Gram-Schmidt's orthogonalization to compute polynomials that would be used to make up the new equation of state. These polynomials are based on a simple equation of state: ideal gas law. A cubic polynomial expansion is fitted to the p-v-T data for ethylene and the results show a reasonable fit. The parameters used are the compressibility of the saturated liquid, the specific volumes of saturated liquid and vapor, and the second virial coefficient, all at the same temperature.

Johanna Ortiz

Hometown: Jersey City, New Jersey

Major: Biology

University: College of Saint Elizabeth

Faculty Mentor: Dr. John F. Cannon, Molecular Microbiology & Immunology

Funded by Summer Undergraduate Breast Cancer Research Program

Identification of Glc7 and its regulator Glc8 in *Saccharomyces cerevisiae*

Johanna Ortiz, Ciprian Crismaru, and John F. Cannon

The main purpose of our study is to evaluate the function of protein phosphatase type 1 (PP1) in the eukaryotic cell cycle. PP1 is an enzyme that ensures faithful chromosomal segregation and regulates kinetochore attachment during spindle assembly. Hence, it is an important factor in the cell cycle checkpoints, particularly in the G2 and M phases. In *Saccharomyces cerevisiae* , or budding yeast, the gene that encodes the catalytic subunit of PP1 is called *GLC7* . Based on prior work, it is known that Glc7 has at least 9 noncatalytic subunits involved in regulating its activity. In this study,we have focused on one noncatalytic subunit - Glc8 in the regulation of PP1.

The techniques that we have used to examine the interactions between Glc8 and Glc7 are both genetic and biochemical. For Glc8 to function it must be phosphorylated by a cyclin-dependent protein kinase called Pho85. Even though Glc8 is not essential for viability, a *glc7-R121K* mutant strain does require Glc8 for growth. Therefore, we expect Pho85 mutations in combination with *glc7-R121K* mutation to be lethal. In an attempt to hunt for these mutants, we will employ a colony-sectoring assay. To further test our hypothesis, we have also disrupted some of the cyclins associated with Pho85 on *glc7-R121K* mutants.

Understanding the structure of Glc8 can allow us to biochemically analyze how it binds and regulates Glc7. Therefore, our target method in elucidating the structure of Glc8 is to purify the protein. Once the protein is successfully purified, we will then analyze its structure using X-ray crystallography. Because these proteins are critical components in the cell division cycle, revealing the interaction between these proteins is important. Thus, gaining insight into the mechanisms associated with the cell cycle checkpoint can pave the way for future implications on therapeutic interventions for cancer.

Shelley Pasternak

Hometown: North Hollywood, California

Major: Biochemistry and Fisheries & Wildlife

University: University of Missouri-Columbia

Faculty Mentor: Dr. John Faaborg, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A comparison of average body condition of nestling Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*) as a determinant of habitat condition in evenaged and unevenaged forest treatments in Missouri Ozark Forest.

Shelley Pasternak, Derek Freund, Salvador Luna-Garcia, and Garrett Rock

We conducted a pilot study in the Missouri Ozark Forest comparing the average body condition of bird species that nest in early succession habitats, Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*). We measured the nestlings when their feathers were just emerging from their shafts. Yellow-breasted Chats were 5-7 days old and Yellow-billed Cuckoos were 2-3 days old. We weighed and measured the tarsus and wing lengths of the birds. We then determined body condition using mass corrected for size. We compared the body condition of birds in evenaged and unevenaged treatments within each species. Yellow-billed Cuckoos showed no significant difference in body condition between the evenaged and unevenaged forest treatments. Likewise, Yellow-breasted Chats showed no significant difference in body condition between the evenaged and unevenaged forest treatments.

Chirag Patel

Hometown: Liberty, Missouri

Major: Chemical Engineering

University: University of Missouri-Columbia

Faculty Mentor: Dr. Tom Phillips, Biological Sciences

Funded by grant to T. Phillips

Light microscopic immunocytochemical localization of DIP organlles in root-tip cells of transgenic tobacco plants

Chirag Patel

The Phillips lab, working in collaboration with the laboratory of John Rogers (Washington State University), has been examining the biogenesis of protein storage vacuoles (PSV) in tobacco and tomato seeds. The PSV contains 3 distinct regions surrounded by a tonoplast membrane: the crystalloid, the globoid, and the matrix. The Phillips and Rogers labs have recently shown that membrane proteins (DIP, RMR and the transgenetic reporter protein Re-F-B-α) are localized to the crystalloid (Jiang, Phiilips, Rogers & Rogers, 2000 J. Cell Biology 150:755-769). They have also shown that the globoid is surrounded by a membrane that contains vacuolar pyrophosphatase and γ-TIP (Jiang et al., submitted). These finding raise an important question of how the membranous components of the crystalloid and globoid are delivered to the PSV and incorporated into this compound organelle.

LM immunocytochemistry has found evidence for a 1-2 μm vesicle in root-tip cells of transgenic tobacco plants. These vesicles were positively stained by antibodies against DIP (dark-induced protein) and are called DIP organnlles. The DIP organelles are also stained by markers of transgenic reporter proteins trafficking to the globoid and crystalloid compartments (Juah, Phillips, and Rogers 1999 Plant Cell 11:1867-1882).

HYPOTHESIS: DIP organelles are multi-vesicular bodies (MVB) formed by membrane traffic direcly from the ER and vesicles derived from the Golgi. These MVB are responsible for delivering both the membranous components fo the globoid and crystalloid to the PSV, as well as souble proteins found in the matrix and globoid.

GOAL: To use light microscopic immunocytochemistry to screen transgenic tobacco root tips to identify plants that have express transgenic reporter proteins and to identify which cells types are actively expressing the DIP and reporter proteins so that electron microscopic immunocytochemistry can be used to examine the structure of the DIP organelles.

Jon Patterson

Hometown: Blue Springs, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Lixing Reneker, Ophthalmology

Funded by Life Sciences Undergraduate Research Opportunity Program

Lens Cell Differentiation in Transgenic Mice Expressing Human Insulin Gene

J. Patterson

The vertebrate lens is composed of two types of cells. A single layer of lens epithelial cells covers the front surface with fiber cells making up the bulk of the lens. During lens development, epithelial cells proliferate at the germinative zone, migrate back, and differentiate into lens fiber cells at the transitional zone. Fiber cell differentiation is then accompanied by the termination of DNA replication, cell division and elongation, and the eventual loss of subcellular organelles, including the nucleus during the later stages of maturation. The normal formation of the lens is accomplished by precise control of cell proliferation and differentiation. The lens grows throughout the mouse's lifetime and if there are defects in the developmental stages, it will not function as a transparent lens. Our research interest focused on the role of growth factors in regulating lens cell proliferation and differentiation. In vitro studies have shown that insulin and insulin-like growth factors (IGF's) can induce lens development in vivo. Dr. Reneker generated 14 transgenic mice that expressed human insulin in the lens. By breeding these founder transgenic mice with the non-transgenic (wild-type) mice, we established eight transgenic lines. The other six founder mice lines did not pass the transgene to the offspring (F1). Transgenic mice from all eight lines displayed ocular defects, with the severity varying from cataracts (cloudy lens) to micropthalmia (small eye). The lens of the embryonic and post-natal eyes were sectioned and stained for defects.

Mark R. Patty

Hometown: Edwardsville, Illinois

Major: Physics

University: University of Missouri-Columbia

Faculty Mentor: Dr. Fred Ross, Physics & Astronomy

Funded by National Science Foundation - REU (MU Research Reactor)

Auditing the resources for laboratory experiments in nuclear physics in the Department of Physics and Astronomy

Mark Patty and Fred Ross

The Department of Physics and Astronomy is in the process of revising and updating its undergraduate laboratory courses. In order to assess the current lab offerings in nuclear physics and to design new ones, we decided to take stock of the instrumentation and the radioactive sources currently stored in our department. This effort required 1) evaluating all detectors (for alpha, beta and gamma radiation) owned by the department, 2) using these detectors to positively identify all radioactive sources housed in the department, and 3) attempting to quantitatively determine the activity (number of radioactive atoms) for each source. This effort allows us to determine what radioactive samples are available for designing new experiments and what equipment is available to make appropriate measurements. In addition, this "audit" of radioactive materials in the department insures that we meet the regulatory guidelines set by the Radiation Safety Program of the UMC office of Environmental Health and Safety.

Maurice L. Penny

Hometown: Durham, North Carolina

Major: Physiology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Meredith Hay, Dalton Cardiovascular Research Center

Funded by National Science Foundation - Access to Doctoral Education

Effects of Nitric Oxide in the Nucleus Tractus Solitarii of Overiectomized Rats

Maurice Penny and Meredith Hay

Post-menopausal women are at greater risk for cardiovascular disease (CVD) and it has been suggested this is due to the loss of estrogen. Studies have shown that CVD such as hypertension and coronary heart disease (CHD) are less common in pre-menopausal women compared to age matched men. This protection in women against CVD disappears with reproductive senescence or on removal of endogenous ovarian steroids. The potential mechanism underlying the effects of estrogen on cardiovascular function are unclear but have been suggested to include effects of lipoproteins, vascular reactivity/nitric oxide synthase (NOS) function and the autonomic nervous system. Previous studies have shown that estrogen up regulates NOS expression in endothelial cells. NOS is also expressed in the central nervous system, and effects cardiovascular function. Microinjections of NOS inhibitors into the nucleus tractus solitarius (NTS) have been shown to increase mean arterial pressure (MAP) and renal synaptic nerve activity (RSNA) in normal rats. It is reasonable to hypothesize that estrogen may alter the function of NOS in the NTS. This study will test the hypothesis that estrogen increases the depressor effect of nitric oxide (NO) in the NTS. To test this hypothesis we will investigate the effects of microinjections of NOS inhibitors (L-NAME and LNMMA) into the NTS on MAP, RSNA, and HR in ovariectomized (OVX) rats with and without estrogen replacement. It is anticipated that in the presence of estrogen will enhance the depressor effect of NO.

Karyn E. Pleasant

Hometown: Oxon Hill, Maryland

Major: Biology

University: Florida A&M University

Faculty Mentor: Dr. Dennis Lubahn, Biochemistry/Child Health

Funded by Summer Undergraduate Breast Cancer Research Program

Regulation of the putative estrogen receptor γ by 17β estradiol

Karyn Pleasant, Peter Ansell, Claudia Espinosa-Nicholas, Joshua Weathers, Monica Weeks, Brian Phillips, and Dennis Lubahn

Estrogens have been shown to have many important regulatory functions in mammals. These functions include the regulation of the immune, reproductive, and cardiovascular systems, as well as bone metabolism. Estrogen exposure is a known risk factor in several human cancers.

Another estrogen receptor, in addition to the known ERα and ERβ, would potentially have major therapeutic implications for cancer, fertility, and osteoporosis. It has traditionally been thought that estrogen acts through these two known receptors, however, estrogen responses have recently been documented that cannot be accounted for by these receptors. Increased lactoferrin mRNA expression has been demonstrated upon treatment with 4-hydroxyestradiol, but not when treated with 17β-estradiol. These results suggest that an estrogen metabolite can upregulate expression of an estrogen responsive gene via a novel pathway independent of ERα or ERβ. Recently, [^{3}H]-4 hydroxyestradiol has been synthesized and it has been shown through ligand binding studies that it binds with a high affinity in a variety of mouse tissues. This binding is not competed by 17β-estradiol or ICI 182,780, but binding competition is observed when 4-hydroxyestrone is added. This shows that [^{3}H]-4-hydroxyestrone and [^{3}H]-4-hydroxyestradiol bind to the same protein. The former is used because it is easier to synthesize in the lab. Previous results have shown that levels of [^{3}H]-4-hydroxyestrone binding vary between ArKO (aromatase knockout) and wild type animals. This suggests that 17β-estradiol might be involved in the regulation of ERγ.

To test this theory, transgenic male mice unable to produce estrogen metabolically (ArKO mice) were treated with 17 β-estradiol and 4-hydroxyestrone. Binding levels in cell extracts from various tissues were compared to the levels in untreated control ArKO male mice. Ligand binding tests were done in the presence of 17β-estradiol to saturate any ERα or ERβ present to prevent binding to the [^{3}H]-4-hydroxyestrone.

Preliminary tests indicate that this potential receptor is regulated by 17β-estradiol in several ArKO male tissues.

Timothy Pouland

Hometown: Lakewood, Colorado

Major: Chemistry

University: Western State College of Colorado

Faculty Mentor: Dr. Douglas D. Randall, Biochemistry

Funded by Plant Genomics Internship at MU

Does recombinant dihydrolipoamide dehydrogenase (E3) increase activity of purified mitochondrial pyruvate dehydrogenase complex (PDC) ?

Timothy Pouland, Nancy R. David, and Douglas D. Randall

Mitochondrial pyruvate dehydrogenase complex (PDC) catalyzes the decarboxylation of pyruvate with the formation of acetyl-CoA and the reduction of NAD+. This complex is located at the entry point for carbon into the citric acid cycle. This multi-enzyme complex is composed of approximately 200 subunits and during purification there is some evidence for dissociation of some components from the complex. Earlier results suggested that the dihydrolipoamide dehydrogenase (E3) component may dissociate and limit maximal activity of the purified complex. My goal was to express and purify recombinant E3 and to determine the amount of E3 required for maximal PDC activity. E. coli BL21(DE3) cells were grown and induced to produce E3. The recombinantly expressed E3 was located in the inclusion bodies requiring solubolization, re-naturation and purification according to M. Faure et al (Eur. J. Biochem. 267: pp. 2890-2898, 2000). The yield of purified, homogenous recombinant E3 was about 2.8 mg from a 250 mL culture . This purified E3 was then used to titrate purified mitochondrial PDC to maximal activity. The results show that a typical preparation of pea leaf mitochondrial PDC is about 36 % deficient of E3. Therefore to quantitate purified PDC it is necessary to reconstitute the complex using recombinant E3.

Thomas Ream

Hometown: St. Louis, Missouri

Major: Biological Sciences

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jim Birchler, Biological Sciences

Funded by Plant Genomics Internship at MU

Finding a molecular explanation for heterosis in maize

Thomas Ream, Don Auger, and Jim Birchler

Heterosis is the phenomenon where hybrid offspring exceed the vigor of both parents from different inbred lines. The classical dominance and over-dominance theories attempt to explain why heterosis occurs, but these ideas tend to ignore the molecular aspects of what happens in heterosis. The overall goal is to determine the types of changes in gene expression, if any, that occur in hybrids relative to inbreds. This information would be useful for formulating a molecular explanation of heterosis.

Our lab developed an interest in this topic from previous studies of dosage dependent regulatory genes. Many regulatory genes are dosage dependent. Of these genes, most act negatively. This means they operate to decrease the amount of gene product the cell would make as the copy number of the regulatory genes increases. We hypothesize that heterodimers of regulatory factors in hybrids, composed of different allelic subunits than their inbred parents, may be less effective in controlling transcription. If this is true, then target gene expression in hybrids will fluctuate compared to the inbred parents. Whether or not expression increases or decreases depends if the regulatory factor acts positively or negatively. Because most regulatory genes act negatively, we expect a net increase in gene expression. Therefore, we postulate that gene products rate-limiting on growth might follow a similar pattern. This would explain why hybrid plants show more vigor than their parents, and how dosage dependent regulatory genes affect expression levels.

In our experiment, we tested levels of gene expression in leaf tissues of hybrid offspring generated from reciprocal crosses between Missouri 17 and B73 inbred lines. In addition, we examined two types of triploid hybrids to test whether any changes in expression were indeed modulated by allelic dosage, which differs in the two triploid hybrids (AAB vs. ABB). We generated these individuals by applying a dose of 0.2% Treflan, a spindle fiber-inhibiting chemical containing the active ingredient Trifluralin, to tassel spikes 8-10 days before flowering. To examine gene expression, RNA was isolated, subjected to Northern analysis and probed to quantitate expression.

Karen E. (Riddle) Trainor

Hometown: Columbia, Missouri

Major: Natural Sciences (Emphasis in Biology and Chemistry)

University: Columbia College

Faculty Mentor: Dr. David Eide, Nutritional Sciences

Funded by Plant Genomics Internship at MU

Identitying genes involved in copper, iron, and zinc ion homeostasis in *Saccharomyces cerevisiae* yeast

Karen (Riddle) Trainor, Amanda Bird, and David Eide

Copper, Iron, and Zinc are essential for the growth of all organisms. Two classes of small proteins, copper chaperones and metallothioneins, are known to have play roles in metal ion homeostasis. These classes are made up of proteins that have small open reading frames (ORFs) and contain many cysteine and histidine residues. The amino acids cysteine and histidine are often involved in coordinating copper, iron and zinc. A screen of the *Saccharomyces cervisiae* yeast deletion project was done to find ORFs that encoded proteins containing 150 amino acids or less and contained at least 8 cysteine or histidine. 36 mutants that met our criteria, were grown up on synthetic complete (SC) plates containing differing levels of the metals for two days. Out of the tested mutants, five produced reproducible growth phenotypes. Some mutants grew better than the wild type (BY4743) on metal deficient plates or not as well as the wild type on metal sufficient plates. The wild type is the *Saccharomyces cerevisiae* strain that does not contain any mutations. The promoter region, ORF, and terminator region of each of the five mutants were then amplified using standard polymerase chain reaction (PCR) methods. The pRS316 vector was cut with *Eco* RI and *Bam* HI enzymes for insertion of our amplified genes. The PCR product was cloned into the digested vector via homologous recombination. Following homologous recombination, plasmids were rescued using the high yield plasmid rescue protocol for yeast. After purification, the plasmids were digested with *Eco* RI and *Bam* HI to see if any contained the PCR insert. Upon conformation of the presence of the gene insert, yeast containing the gene clone were screened to see if introduction of the gene complemented the growth phenotype of the mutant.

Garrett L. Rock

Hometown: St. Joseph, Missouri

Major: Biological Sciences

University: University of Missouri-Columbia

Faculty Mentor: Dr. John Faaborg, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

A comparison of average body condition of nestling Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*) as a determinant of habitat condition in evenaged and unevenaged forest treatments in Missouri Ozark Forest

Shelley Pasternak, Derek Freund, Salvador Luna-Garcia, and Garrett Rock

We conducted a pilot study in the Missouri Ozark Forest comparing the average body condition of bird species that nest in early succession habitats, Yellow-breasted Chats (*Icteria virens*) and Yellow-billed Cuckoos (*Coccyzus americanus*). We measured the nestlings when their feathers were just emerging from their shafts. Yellow-breasted Chats were 5-7 days old and Yellow-billed Cuckoos were 2-3 days old. We weighed and measured the tarsus and wing lengths of the birds. We then determined body condition using mass corrected for size. We compared the body condition of birds in evenaged and unevenaged treatments within each species. Yellow-billed Cuckoos showed no significant difference in body condition between the evenaged and unevenaged forest treatments. Likewise, Yellow-breasted Chats showed no significant difference in body condition between the evenaged and unevenaged forest treatments.

Mario Antonio Saldana Torres

Hometown: San Juan, Puerto Rico

Major: Civil Engineering

University: Polytechnic University of Puerto Rico

Faculty Mentor: Dr. John Bowders, Civil and Environmental Engineering

Funded by National Science Foundation - Access to Doctoral Education

Design, Development and Implementation of a Model for Teaching the Importance of Foundations for Structures

Mario A. Saldana Torres, John Bowders, Erik Loehr, Hani Salim, and Rick Wells

In Civil Engineering practice, foundations are used to transfer the loads of structures to competent soil or rock. The performance characteristics of the foundation ultimately determine the response of the structure. It is important for the Civil Engineer to understand this critical interrelationship. In order to help Civil Engineering students grasp and learn the principals of soil-structure interactions and to explain the significance of the foundations of structures a physical model of a structure and its foundation system was constructed. This model will be used to demonstrate how the responses of the structural members of a building may vary according to the foundation and loading conditions. The model structure consists of a welded-bolted two-level, three bay steel frame in which the foundation stiffness beneath each column can be varied. The member sections were sized accordingly as determined by a structural analysis performed using the computer software RISA-3D to check for structural stability. The foundation system consists of foam of varying stiffnesses. These can be changed to yield different responses by the structure when placed under load. Sixteen strain gages are being used to allow for physical measurement of the strains (movement) in the steel members when the structure is loaded. The strain gages have arrived and will be attached through a tedious process. Once the strain gages are attached, the model will be put through a series of test loading conditions, and predicted strain will be compared with measured strains. Measured strains under different conditions will be compared to theoretical values.

Charcacia T. Sanders

Hometown: Dallas, Texas

Major: Biology

University: Prairie View A&M University

Faculty Mentor: Dr. Grace Sun, Biochemistry

Funded by National Science Foundation - REU (Life Sciences)

Genistein and quercetin inhibit(s) nitric oxide produced by activated microglial cells

Charcacia T. Sanders, Qun Zheng, and Grace Sun

Nitric oxide (NO) is a signaling molecule that acts in many tissues to regulate a diverse range of physiological processes. It is synthesized by L-arginine by NO synthase. NO has been associated with cell death in neurodegenerative diseases, infection, and brain injury such as Parkinson's disease, Alzheimer's disease, and stroke. Induction of brain tissue inflammation involves a widespread activation of glial cells, such as microglial cells, and subsequent release NO, which is involved in increasing oxidative stress to the cells. Pro-inflammatory agents such as endotoxins (lipopolysaccharide, LPS) and interferon-γ (IFN-γ) can actively induce the production of NO in microglial cells through induction of the inducible NOS gene. Flavonoids, such as genistein and quercetin, are naturally occurring antioxidants found in vegetables, fruits, and plants. Genistein is enriched in soybean and is regarded as a phytoestrogen.

The purpose of this experiment is to investigate whether flavonoids such as genistein and/or quercetin can inhibit NO production in microglial cells that are stimulated by cytokines.

Experimental procedures: Mouse BV-2 microglial cells were grown in 35mm dishes to confluence. In the first study, BV-2 cells were stimulated with different concentrations of INF-γ and LPS to find the most effective concentrations for induction of NO release. After treatment with cytokines for 16 hr, the culture medium was taken to determine NO concentration (as nitrite) using the Greiss reagent. Cells were removed from dishes for protein determination. In some instances, morphological changes were observed under a Nikon inverted microscope. To test for effects of flavonoids, BV-2 cells were treated with different concentrations of genistein and quercetin and incubated for 30 minutes prior to adding IFN-γ (10ng/ml) or LPS (50ng/ml) and further incubated for 16 hours.

Results: BV-2 cells respond well to IFN-γ but less well to LPS in the induction of NO. Treatment with IFN-γ resulted in morphological changes in these cells. Both genistein and quercetin dose-dependently inhibited NO production induced by IFN-γ . Inhibition by genistein appeared to reverse morphological changes induced by IFN-γ . RT-PCR was carried out to examine whether genistein and quercetin inhibited NO production through inhibiting NO production through inhibiting the mRNA of iNOS gene.

This study suggest that a diet, which include foods or dietary supplements containing high concentrations of genistein or quercetin, can be helpful to reduce or prevent oxidative stress cause by NO production.

Kimberly Sartain

Hometown: Springfield, Missouri

Major: Biology

University: Southwest Missouri State University

Faculty Mentor: Dr. Fred Vom Saal, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Effects of soy on male development

Kimberly Sartain, Rachel Ruhlen, Catherine Sandner, and Fred Vom Saal

Plants contain many compounds with estrogenic activity, which are collectively called phytoestrogens. There is interest in soy as a source of phytoestrogens. Soy has become commonly used as a dietary supplement and in baby formula exposing humans to much higher levels of phytoestrogens then would naturally occur from eating foods containing soy. During aging soy may have some beneficial effects such as reduced rates of osteoporosis, prostate and breast cancer, but little research has been done on the effects of soy on development. This study looked at the effects of soy on male hormone levels, reproductive organs, rate of growth, and body fat. Prior to pregnancy a group of female mice were fed a high soy diet and another group were fed a medium soy diet. The offspring were maintained on their respective diets until they reached three months of age, at which times the males were examined. Body weight, the two major regions containing fat (the gonad and renal fat pads), reproductive organs (testes, prostate, seminal vesicles), liver, spleen and kidney were weighed. Serum estradiol was measured by radioimmunoassay (RIA). Daily sperm production was determined using a hemacytometer. Animals maintained on medium soy diet had significantly more body fat (gonadal and renal fat pads) then animals on the high soy diet. Since there was no difference in body weight this suggests other differences in body composition. The testes were significantly larger and daily sperm production tended to be higher on the medium soy diet. The prostate was also somewhat larger on the medium soy diet but the difference was not significant. In contrast the animals on the high soy diet had larger kidneys and spleen. The differences seen could be due to increased levels of endogenous estradiol in the animals fed a high soy diet, which contains higher levels of phytoestrogens. The total estrogen exposure of animals on a high soy diet was greater then the levels provided through the diet alone.

Charlotte Schnellbacher

Hometown: St. Louis, Missouri

Major: Biology and Anthropology

University: Truman State University

Faculty Mentor: Dr. Tobias Baskin, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

Investigation into the cellular structures involved in plasmolysis

Charlotte A. Schnellbacher and Tobias I. Baskin

When a plant cell is placed in a hypertonic solution, the phenomena of plasmolysis occurs, in which the plasma membrane pulls away from the cell wall as water is lost to the surrounding solution through osmosis. Contrary to expectation, the cell does not round up into a sphere or an oval. Some regions of the cell appear to stick to the cell wall while other regions retract, giving the plasmolyzed cell a complex shape. The reason for this heterogeneity is not understood. The primary objective of this study is to identify the cellular structures responsible for the shapes of plasmolyzed cells.

Initially, we needed to develop a technique for imaging plasmolysis. Seedlings of *Arabadopsis thaliana* were grown on agar plates for 7-9 days. To plasmolyze cells, seedlings were transferred to a small volume of hypertonic sucrose solution on a microscope slide and immersed for 20 min. The sucrose solution contained 3,3 dihexlyoxacarbocyanine iodide (DIOC), a fluorescent dye known to stain the plasma membrane. Preliminary experiments showed that a DIOC concentration of 20 μM gave consistently bright results. Stained cells were imaged with fluorescence microscopy. To document findings, images were captured with a video camera, interfaced to the microscope, digitized with image processing software, and saved to disk. Figures were prepared for presentation with Adobe Photoshop.

With clear staining of the plasma membrane, we found that the shapes of the plasmolyzed cells in arabadopsis roots were complex and, between 0.5 and 1.5M sucrose, the kinds of shapes formed depended on concentration. We attempted to confirm a plasmolysis deficit previously inferred by the Baskin lab in the mutants *rsw4* and *rsw7*, but were unable to do so, suggesting that the previous inference was incorrect. Cell shape is often regulated by the cytoskeleton. To examine the role of microtubules, they were depolymerized with 1 μM oryzalin for 2-24 h before plasmolysis. The shapes of the plasmolyzed cells following oryzalin treatment did not differ systematically from untreated cells suggesting that microtubules do not dictate plasmolysed cell shape. Conversely, inhibiting actin with 10-40 nM latrunculin-B for 2-3 h, resulted in more severe forms of plasmolysis at lower sucrose concentrations. We conclude that the actin plays a role in sculpting the plasmolyzed cell.

Brandi L. Schottel

Hometown: Frisco, Texas

Major: Chemistry and Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jerry Atwood, Chemistry

Funded by Life Sciences Undergraduate Research Opportunity Program

Investigation of snub cube formation for specific drug delivery systems

Brandi L. Schottel, Charles L. Barnes, and Jerry Atwood

Initial investigations into the formation of a snub cube precursor, $C_{56}H_{48}O_{20}$, are presented. Pyrogallol and vanillin, in the presence of acid, form C-4-hydroxy-3-methoxyphenyl-resorcin[4]arene. Crystals grown of this precursor from methanol and nitrobenzene reveal the tetramer structure required [triclinic,P-I, a=10.976, b=13.0235, c=13.4978A, α=62.16, β=81.727, γ=65.477°, Z=1, density calculated=1.1156g/cm^3, T=173.15K, Siemens SMART CCD diffractometer, $Mo_{K\alpha}$, (wavelength= 0.71073).] Initial mass spectral analysis suggests the presence of the snub cube formed from six precursors.

S. Nicole Schultz

Hometown: Columbia, Missouri

Major: Nursing

University: University of Missouri-Columbia

Faculty Mentor: Dr. Jane Armer, Nursing

Funded by McNair Scholars Program

Nursing measurements of lymphedematous limbs

S. N. Schultz, Jane Armer, Deidre Wipke-Tevis, and Donna Williams

There is lack of precision in measuring limb fluid volume changes associated with post breast cancer lymphedema which hinders early detection and evaluation of effective treatment. Our current research project compares 2 instruments, the perometer and the tape measure, to the "gold standard" of water displacement to determine which measures most accurately and reliably. Preliminary findings based on data from healthy volunteers (n = 4) suggested that the circumferential measurement more closely estimates the water displacement volume than the perometer, with the perometer consistently overestimating the water displacement volume

Teresa Sharillo

Hometown: Colchester, Connecticut

Major: Ecology and Evolutionary Biology

University: University of Connecticut

Faculty Mentor: Dr. John Faaborg and Dr. Paul Porneluzi, Biological Sciences

Funded by Missouri Ozark Forest Ecosystem Project

Use of Clearcuts by Forest Breeding Bird Species

Teresa C. Sharillo and Marie T. E. Abbott

The purpose of this data analysis is to determine whether forest breeding bird species are using clearcuts, which are a type of timber harvesting forest treatment. The importance of this analysis is to see if clearcutting, which is a loss of breeding habitat for forest breeding birds, serves other purposes for these species. To conduct this analysis we used mist-netting data that sampled the bird species within and around the clearcuts during the month of July. To determine whether forest birds were defending territories in the clearcuts we used data from spot maps collected in late May through June. We sampled thirteen clearcuts of varying size, ranging from 9.3 hectares to 0.772 hectares, in Southeastern Missouri that were harvested in 1996. From these data we determined the number and age of forest species found in the clearcuts. We concluded from the analysis that the forest birds are using the clearcuts during the month of July but were not defending territories in late May and June. We also found that many of the forest breeding species caught in the mist-nets were hatch year birds, suggesting that the most likely use of the clearcuts for forest species is to protect their fledged offspring from predators and to utilize a more abundant food supply.

Saraia S. Smith

Hometown: Collinsville, Illinois

Major: Biological Engineering

University: St. Louis Community College at Florissant Valley

Faculty Mentor: Dr. Allen Thompson, Biological Engineering

Funded by National Science Foundation - Access to Doctoral Education

The Effects of PAM Application Method with the Influence of Kinetic Energy on Infiltration and Erosion

Saraia Smith, F. Ghidey, and Allen Thompson

Previous research has indicated that polyacrylamide (PAM) soil amendments can be effective in reducing runoff volume and soil erosion by reducing sealing and stabilizing soil structure. Furthermore, the effect of raindrop kinetic energy (KE) on infiltration, and subsequent runoff and sediment transport from a silt loam claypan soil with the addition of PAM has been shown to reduce runoff volume and sediment yield on some soils. A laboratory study was conducted using simulated rainfall to test the effectiveness of the method of PAM application (dry and aqueous) onto dry soil. The rainfall simulator is a stationary, gravity fed unit consisting of 906 individual drippers arranged in an equilateral triangular arrangement with individual drippers 3.85 cm apart. The simulator is capable of producing application rates up to 24 cm/h. Tests were conducted at 6.4 cm/h, with the droplet inceptor screen positioned 0.47 m below the drippers producing droplet sizes very similar to natural rainfall at that intensity. Results indicated that sediment loss was directly related to KE. The addition of 19.76 kg/ha (8 lb/ac) PAM reduced both soil loss and total runoff volume compared to the bare soil plots with no PAM.

Douglas B. Snider

Hometown: Princeton, Missouri

Major: Animal Science

University: Southwest Missouri State University

Faculty Mentor: Dr. Donald Spiers, Animal Sciences

Funded by F.B Miller Undergraduate Research Program in Animal Sciences

Development of a basic model to predict rectal temperature in cattle based on telemetry measures of thermal status

Douglas B. Snider, Donald E. Spiers, and Hosam Al-Tamimi

A cattle study was designed to determine relationships between rectal temperature and more centralized regions that include both rumen and peritoneal cavity. Rectal temperature has traditionally been used for determining the body temperature of cattle during heat stress. Using a state-of-the-art telemetric thermometer device (Model BV-010, CowTemp-Bovine Health Monitoring System, Innotek Wireless Sensor Division, Garrett, IN), we now have the ability to monitor thermal status in many different regions of the body. Likewise, we will be able to monitor animals in a variety of conditions without risking injury and stress caused by handling. This will aid environmental stress research, where monitoring a large number of animal body temperatures is necessary to assess thermal status. Therefore, a controlled study is needed to compare different measurements of body temperature for assessment of thermal status under a variety of conditions, using present and past techniques. In addition, basic thermoregulatory differences of these three sites can be examined in this preliminary study. Three steers (259 ± 6.9 kg BW) were used throughout thermoneutral and heat stress periods. Using environmental chambers (Brody Environmental Center, A.R.S.C., UMC), animals were exposed to constant thermoneutral conditions (TN) for 4 days at 19*C and cycling heat stress conditions (HS) for 17 days at 36 ± 5*C. Cycling conditions during heat stress included 26*C from 0000 to 0400, an increase to 36*C at 1200, constant 36*C from 1200 to 1600, and decrease back to 26*C at 0000. Body core temperatures (rumen and peritoneum) were measured at 8-12 minute intervals using telemetry. Rectal temperatures were measured at 0800, 1100, 1600, and 2000 using a thermistor probe (Model 8110-20, Cole-Parmer Instrument Company, Chicago, IL). Monitoring circadian changes in body temperature provide indications of when the body is most affected by heat stress. Highest linear correlation coefficients with rectal temperature were for mean peritoneal, maximum peritoneal, and maximum rumen temperatures (r=0.69-0.97) during all phases of study. Correlations between these variables and air temperature were much less (r=0.02-0.39). Peritoneal temperature was higher than ruminal temperature in linear correlations with rectal and air temperatures. However, maximum rumen temperature may be the more practical measure, since initial thermometer placement is relatively harmless and the temperature changes in this region reflect daily patterns of drinking activity. Such activity is important in the assessment of thermal stress in the field. It was interesting to note that linear correlation coefficients and slopes of relationships between rectal temperature and both peritoneal and rumenal temperatures increased from thermoneutral through mid-heat phases of the study (i.e., r: 0.86 > 0.95; slope: 0.87 > 1.131), to suggest increased responsiveness. In contrast, both components of the linear relation decreased into the last period of HS at 17 days, to indicate potential adaptation and decreased predictive ability at this point. Future field studies must be performed to refine the model and determine the effect of other environmental factors (e.g., wind speed, relative humidity, and radiant heat) on these relationships.

Laura Sullivan

Hometown: Tulsa, Oklahoma

Major: Biology (Fish and Wildlife Management)

University: Northeastern State University

Faculty Mentor: Dr. Mia Revels, Biological Sciences, Northeastern State University

Funded by Missouri Ozark Forest Ecosystem Project

Determining the territorial ranges of blue-winged warblers as affected by the cardinal direction of the specified habitat

Lesley Avery and Laura Sullivan

Our intended purpose of this study is to locate the approximate territories and overlap of territories of blue-winged warblers with respect to cardinal direction and vegetation of regenerating clearcuts in the Mark Twain National Forest. Blue-winged warblers were chosen because they are an edge species that are becoming more abundant in the study site.

Having combined data collected in previous years with that collected this year, we have noticed various trends including increased presence of blue-winged warblers five years after the cutting occurred and the difference in abundance of blue-winged warblers based on cardinal direction of the clearcut on the slope of the mountain. Territories and approximate density populations were detemined via mist netting and spot-mapping in and along clearcuts.

Katharine A. Swoboda

Hometown: Lincoln, Nebraska

Major: Biology

University: University of Nebraska-Lincoln

Faculty Mentor: Dr. Arun K. Chatterjee, Plant Microbiology and Pathology

Funded by Plant Genomics Internship at MU

Effects of *flhCD* $_{Ec153}$ on growth and exoenzyme production in *Erwinia carotovora* subsp. *carotovora* strain Ecc71 at elevated temperature

Katharine A. Swoboda, Hiroaki Hasegawa, Yaya Cui and Asita Chatterjee

Erwinia carotovora subsp. *carotovora* (Ecc71) produces exoenzymes that cause soft rot in potato, celery, and cabbage. These extracellular pectate lyases (Pel), polygalacturonases (Peh), proteases (Prt) and cellulases (Cel) cause tissue maceration and cell death. Exoenzyme production in Ecc71 is controlled by several regulator genes including *flhCD* and influenced by temperature. Ecc71 exhibits slow growth and reduced enzyme production at elevated temperatures, whereas *Erwinia carotovora* strain 153 (Ec153) grows exponentially and produces enzyme at elevated temperatures.

Experiments were performed to determine whether the regulation of *flhCD* is temperature sensitive in Ecc71 and if the presence of *flhCD* $_{Ec153}$ in Ecc71 influences its growth and enzyme production. Results indicate that the presence of *flhCD* $_{Ec153}$ does not influence growth in Ecc71. Enzyme assays indicate that exoenzyme production is dependent upon temperature in both wild type and Ecc71*flhCD*- strains and is slightly elevated in the presence of *flhCD* $_{Ec153}$. Northern hybridization results indicate that the expression of *flhCD* is not dependent upon temperature and that the production of pectate lyase (Pel) is slightly elevated in the presence of multi-copy *flhCD* in both wild type and *flhCD*- strains. Southern hybridization and polymerase chain reaction (PCR) results reveal the presence of *flhCD* homologues in other *Erwinia* species. In the future, similar experiments should be carried out in other species of *Erwinia* to determine the validity of these results.

Aaron Tesfai

Hometown: Jefferson City, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Sheryl Tucker, Chemistry

Funded by Life Sciences Undergraduate Research Opportunity Program

Spectroscopic Comparison of Amine-Terminated PAMAM and PPI Dendrimers

Aaron Tesfai, Dana L. Richter-Egger, and Sheryl A. Tucker

The physical and chemical properties of amine-terminated PPI and PAMAM dendrimers' interiors were investigated using the fluorescent, solvatochromic probe phenol blue. In aqueous solutions of each generation studied, two discrete dye populations were clearly observed. Both the amine-terminated PPI and PAMAM dendrimers were shown to form tight, nonpolar associations with the majority of the available dye, within the dendrimer interior, near the core. In the absorption and steady-state fluorescence emission spectra, a microenvironment of decreasing polarity in increasingly larger-generation PAMAM and PPI dendrimers (up to G3) is seen for the associated probe. The remaining larger-generation dendrimers (>G3) all provide a microenvironment of essentially equal polarity. Model compounds that mimic PAMAM's and PPI's surface groups and branching moieties were used to better define the associated dye's location. The mimics further confirm that phenol blue is associated inside the dendrimer, where it does not interact with the amine dendrimer surface groups. The comparison of amine-terminated PPI and PAMAM dendrimers clearly demonstrated the effects of their structural differences and the ability of phenol blue to have sensed those differences-including the initiator core and branching unit length and branching unit chemical composition.

Darla Lynn Tharp

Hometown: Dixon, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Kerry S. McDonald, Physiology

Funded by McNair Scholars Program

Effect of mechanical overload on functional properties of mouse plantaris muscle

Darla L. Tharp, Richard W. Tsika, and Kerry S. McDonald

Mammalian skeletal muscle is needed for the production of force and movement of organisms. Skeletal muscle is highly plastic in that it responds to activity or inactivity by increasing or decreasing in size. One model used to study an increase in muscle size (i.e., muscle hypertrophy) is the mechanical overload (MOV) model. Mechanical overload involves surgical removal of plantar flexor muscles such that only the plantaris muscle bears load. Few studies have measured the functional properties of overloaded muscle and, thus, was the first aim of this study. To observe the effect of mechanical overload on the functional properties of the plantaris, a comparison of force frequency and fatigue relationships of control versus MOV mice was determined. The second aim of the study was to investigate cellular signaling pathways that may induce hypertrophy in response to overload. The putative pathway of focus is the calcineurin-signaling pathway, specifically focusing on the downstream regulator known as nuclear factor of activated T cells or NFAT. Using NFAT4 knockout mice, we also measured force frequency and fatigue relationships to determine the function of NFAT4 in mechanical overload induced hypertrophy. Mechanically overloaded mouse plantaris muscle exhibited marked hypertrophy, a slight rightward shift in force frequency relationships, and a decrease in fatigability. Also, preliminary results suggest that the genetic removal of NFAT4 isoform attenuates hypertrophy but does not prevent the changes in force-frequency and fatigability normally associated with MOV in mouse hindlimb skeletal muscle.

Jarron I. Tilghman

Hometown: St. Peters, Missouri

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Stephen Alexander, Biological Sciences

Funded by Summer Undergraduate Breast Cancer Research Program

Cross-resistance to chemotherapeutic drugs: Does resistance to one drug confer resistance to other drugs?

Jarron I. Tilghman, Guochun Li, Hannah Alexander, and Stephen Alexander

Each year, a considerable portion of the population is diagnosed with some form of cancer. These malignancies are caused by mutations in genes that encode proteins responsible for regulating cell growth. Inevitably, excessive proliferation is the result. To counteract this response, most tumors are treated via chemotherapy, radiation therapy, or both. Unfortunately, these tumors often become resistant to chemotherapy. But why? Various mechanisms can account for decreased sensitivity to these drugs (e.g. lowered concentration of drug entering the cells, inactivation of drugs in the cells, increase in DNA repair, or inactivation of cell death). These also arise from mutations in specific genes. A previous study in our lab involved a large mutagenesis experiment for which cisplatin resistance was selected. Six strains were found to be resistant to the drug including one with a mutation in the S-1-P lyase gene. S-1-P lyase catalyzes the catabolism of sphingosine-1-phosphate into hexadecanal and phosphoethanolamine in the sphingomyelin degradation pathway. Removal of this enzyme (as in our mutant strain, S-1-P lyase$^-$) results in an increase in sphingosine-1-phosphate concentration. Reports in the literature suggest that an increase in ceramide concentration promotes cell death, while an increase in sphingosine-1-phosphate concentration yields cell survival. Thus, we think that an increase in sphingosine-1-phosphate in our mutant results in increased survival after cisplatin treatment. Our question then is: "Are strains that exhibit resistance to cisplatin also resistant to other drugs?" To test this, we used the cisplatin-resistant mutant S-1-P lyase$^-$ and checked for resistance to mitomycin and carboplatin.

Rachel Walsh

Hometown: Kansas City, Missouri

Major: Chemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Michael A. Harmata, Chemistry

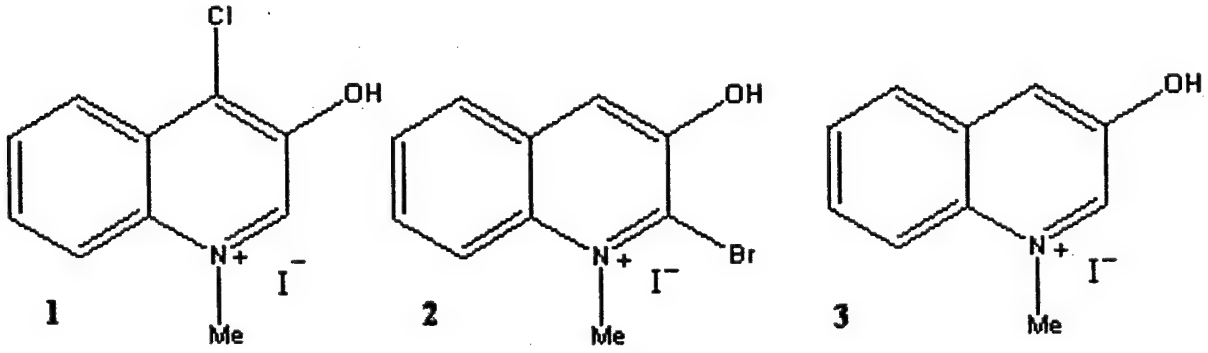
Funded by Life Sciences Undergraduate Research Opportunity Program

4+3 cycloaddition reactions of oxidoquinolinium ions

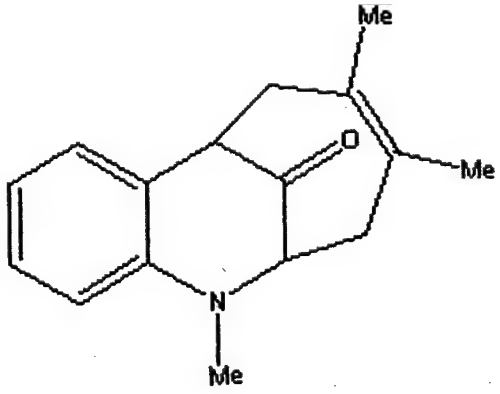
Rachel M. Walsh and Michael A. Harmata

Cycloaddition reactions are powerful tools for the construction of organic molecules with anti-tumor, anti-inflammatory, and anti-bacterial properties. With the goal of devising a procedure for the synthesis of biologically active carbazoles, we undertook a study of the 4+3 cycloaddition reactions of oxidoquinolinium ions.

The proposed synthetic scheme required the incorporation of a halogen atom into the oxidoquinolinium ion. The salts N-methyl-4-chloro-3-hydroxyquinoline (**1**) and N-methyl-2-bromo-3-hydroxyquinoline (**2**) were prepared and examined in a 4+3 cycloaddition reaction with 2,3-dimethylbutadiene. Unfortunately, no cycloaddition products were observed.



In order to determine the effect of the halogen atom in **1** and **2** we undertook a study of similar cycloaddition reactions in the salt N-methyl-3-hydroxyquinoline (**3**). Reaction of **3** with 2,3-dimethylbutadiene results in the following cycloadduct.



Sarah Warren

Hometown: Mount Pleasant, Iowa

Major: Biochemistry and English (Rhetoric and Professional Communications)

University: Iowa State University

Faculty Mentor: Dr. David Eide, Nutritional Sciences

Funded by Plant Genomics Internship at MU

Mutational analysis of zinc induced ZRT1 endocytosis

Sarah Warren, Monir Shababi, and David Eide

Zinc is an essential mineral for cells because it is involved in many protein structures as well as gene transcription and enzyme reactions. In yeast, the ZRT1 protein, located in the plasma membrane, uptakes zinc when intracellular zinc levels are limited. Once the cell has sufficient amounts of zinc, it must degrade ZRT1 protein to prevent toxic zinc levels from accumulating. It does this through the process of endocytosis. If we can genetically interfere with the cell's ability to endocytose the ZRT1 protein, that mutation can be identified in the yeast genome. We will then know which genes are responsible for a specific phase in ZRT1 protein degradation. To induce this mutation, we transformed yeast cells with transposon plasmids inserted randomly into the genome, then screened the resulting population to find the cells with mutations in the endocytic pathway. Currently, we have twelve mutants from which we will remove the originally inserted plasmid and insert another plasmid that contains an origin of DNA replication. This will allow the DNA to be replicated in E. coli, and from there the plasmid DNA will be extracted and sequenced. The regulation of proteins by endocytosis is a phenomenon seen in a variety of other eukaryotic organisms. It is also analygous to processes that occur to hormone and growth factor receptors. Therefore, if we can better understand the regulated endocytosis of ZRT1in yeast, we can then use it as a model for exploring similar pathways in other organisms and other receptors.

Timothy Wertin

Hometown: Elllisville, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Joe Polacco, Biochemistry

Funded by Plant Genomics Internship at MU

Isolation of mitochondrial transporters of arginine from soybean (*Glycine max*)

Timothy Wertin, Elizabeth Hoyos, and Joe Polacco

Arginine (Arg) is an important nitrogen-rich amino acid in soybean seeds. When the seed germinates Arg is released from reserve proteins and must enter the mitochondrion to be converted to other nitrogenous compounds. Hence control of Arg movement through the mitochondrial membrane is important in the regulation of N storage and utilization in the soybean embryo. Two putative mitochondrial Arg transporters have previously been isolated and cloned from *Arabidopsis thaliana* : a carnitine/acetylcarnitine transporter (CAT) and a mitochondrial transporter (MT) with no assigned substrate preference. These transporters correct a yeast mutant, arg11, defective in mitochondrial exchange of Arg and ornithine. *In vitro* , MT catalyzes the movement of Arg into artificial vesicles.

Arabidopsis mitochondrial transporter sequences were used to isolate sequences from *G. max* by BLASTing soy EST databases. Certain conserved regions of mitochondrial transporters have been found within the recovered soybean sequences (which are also present in CAT and MT from *Arabidopsis*), affirming that they are Arg mitochondrial transporters. Yeast expression and *in vitro* vesicle studies will determine the functionality of the soybean sequences. Eventually, they will be modified to produce high-Arg soybeans in transgenic lines.

Chris Wheatley

Hometown: Arnold, Missouri

Major: Biochemistry

University: University of Missouri-Columbia

Faculty Mentor: Dr. Marc Hamilton, Veterinary Biomedical Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Lipoprotein lipase suppression in skeletal muscle after acute physical inactivity, chronic physical inactivity, and tenotomy

Christopher D. Wheatley, Warren T. Cooper, Lionel Bey, Andrea Sano, Enas Areiqat, and Marc T. Hamilton

Lipoprotein lipase (LPL) is a pivotal enzyme in fatty acid and lipoprotein metabolism. As such, it is a key enzyme for study in order to elucidate the mechanisms behind disease processes and other metabolic disorders. LPL removes triglycerides (TG) from chylomicrons and VLDL by lipolyzing them to fatty acids for oxidation in muscle or storage in adipose tissue. These liberated fatty acids are also involved in transcriptional events, cellular signaling and post-translational regulation of multiple genes. Therefore, LPL activity is a measure of the rate of release of these fatty acids from chylomicrons and VLDL. In order to study the effects of physical inactivity we made use of soleus (SOL) muscle—a red postural muscle that is heavily recruited for ambulatory activity. In 10-hour chronic hind-limb suspensions for 11 days there was a profound decrease (67%) in soleus LPL activity without atrophy of the muscle compared to control. However, to determine if this was a local or systemic effect we also assayed white quadriceps (WQ)—a group of white muscles that is not recruited for ambulatory activity. There was no decrease in WQ LPL activity during 12-hour chronic hind-limb suspension. To study the effect of time on the reduction of LPL activity with physical inactivity we acutely suspended rats. Acute 12-hour hind-limb suspension resulted in an even larger decrease (>90%) in soleus LPL activity compared to control. WQ did not decrease with acute suspension, and in addition we assayed diaphragm tissue as it is a red muscle used in breathing and contracts heavily like the soleus. Diaphragm also had no change with acute 12-hour hind-limb suspension, meaning that hind-limb suspension effects only local contractile activity. In order to obtain the best control possible for physical inactivity, we used tenotomy. Tenotomy involved cutting the Achilles tendon near the ankle, thereby eliminating use of the soleus muscle. This resulted in a large decrease (92%) in soleus activity compared to the contralateral (intact tendon) leg. These models of the deterioration of lipoprotein metabolism in skeletal muscle will be helpful for understanding processes in cardiovascular disease, inactivity (especially spaceflight), diabetes, exercise, and an insight into the molecular mechanisms behind these changes.

Andrew A. Wheeler

Hometown: Bolivar, Missouri

Major: Nutritional Sciences

University: University of Missouri-Columbia

Faculty Mentor: Dr. Leona J. Rubin, Veterinary Biomedical Sciences

Funded by Life Sciences Undergraduate Research Opportunity Program

Effect of Exercise Training and High Fat Diet on Uncoupling Protein-2 (UCP-2) Gene Expression in Porcine Vascular Tissues

Andrew A. Wheeler and Leona J. Rubin

Uncoupling proteins, found in the inner mitochondrial membrane, are responsible for uncoupling oxidative phosphorylation by moving protons across the mitochondrial inner membrane toward the mitochondrial matrix and thus disrupting the proton motive force resulting in a net release of heat, rather than ATP being produced. A family of uncoupling proteins is known and consists of three different isoforms, UCP-1, UCP-2 and UCP-3. UCP-2 is found in a wide variety of tissues and was of particular interest to us because of its possible implications in exercise and hyperlipidemic states where its control of ATP formation may be altered. Specifically, fatty acids may be required for increased uncoupling protein activity and at least UCP-2 gene expression appears to be regulated by fatty acids in human skeletal muscle. Exercise is of particular interest in the regulation of vascular tissue UCP-2 expression because of the ability of exercise to clear fatty acids from the blood. To date, neither the identification nor the regulated expression of UCPs has been examined in vascular tissues. We hypothesized that exercise training and/or a diet high in fat would alter expression of UCP-2 in vascular tissues. This hypothesis was tested using a porcine model of exercise training. Pigs exercised on a treadmill for 16 weeks, 5 days per week. Half of these pigs are fed a diet supplemented with fat, primarily palmitate, for 20 weeks (4 weeks prior to and during exercise training). Following the experimental protocols, tissues were collected from skeletal muscle [anterior head of triceps and deltoid], heart muscle [right ventricle], liver, and vascular smooth muscle [coronary and carotid] of sedentary (SED), exercise trained (EX), high-fat fed SED (HF-SED), and high-fat fed EX (HF EX) Yucatan pigs. Total RNA was isolated, reverse-transcribed and PCR was used to identify the gene of interest. Real-time PCR was used to quantify UCP-2 expression using 18S RNA as the internal control. We found that exercise training significantly decreased UCP-2 expression in coronary arteries and liver, with no significant difference between HF EX and EX. UCP-2 expression tended to increase in skeletal muscle of exercise trained animals (EX and HF EX) but did not reach statistical significance: these tissues exhibited biochemical signs of training (increased citrate synthase activity). These results suggest that the regulation of UCP-2 expression by exercise training and/or a high fat diet is tissue specific and may play a significant role in regulation of mitochondrial processes beyond that of ATP synthesis. Speculation regarding the role of UCP-2 actions includes regulation of reactive oxygen species and fatty acid metabolism.

Taiya M. J. Williams

Hometown: Houston, Texas

Major: Biology

University: Prairie View A&M University

Faculty Mentor: Dr. Miriam Golomb, Biological Sciences

Funded by National Science Foundation - REU (Life Sciences)

MLST reveals that invasive nontypeable *Haemophilus influenzae* are clonally related to common respiratory strains

Taiya M. J. Williams, Vanessa Kuwajima, Arnold L. Smith, and Miriam Golomb

Haemophilus influenzae is a gram-negative bacterium commonly found in the upper respiratory tract of humans. Even though *H. influenzae* usually coexists peacefully with its human hosts, some strains cause conjunctivitis, otitis media, meningitis and septicemia. Non-encapsulated, non-typeable *H. influenzae* (NTHi) is normally linked to respiratory diseases, while invasive diseases such as meningitis are generally caused by type b encapsulated *H. influenzae* (Hib). The Hib vaccine has greatly decreased the number of *Haemophilus* meningitis cases in the industrialized world. However, occasional cases of invasive disease linked to NTHi have raised concern about the potential emergence of vaccine-resistant *Haemophilus* meningitis. The purpose of this study was to use MLST (Multi- Locus Sequence Typing) to determine how invasive NTHi strains are related to respiratory strains. Previous studies had suggested a clonal relationship between R2866, a virulent NTHi isolated from a meningitis patient, and two respiratory isolates. MLST confirmed this hypothesis, and showed that isolates from Brazilian purpuric fever, a highly lethal septicemia which emerged in the late 1980's, are clonally related to conjunctivitis strains. Clonality among pathogenic NTHi strains implies recent emergence and adaptive success, and suggests that invasiveness is easily acquired.

Karen Williams

Hometown: Gaylord, Michigan

Major: Biology

University: University of Missouri-Columbia

Faculty Mentor: Dr. Rainer Glaser, Chemistry

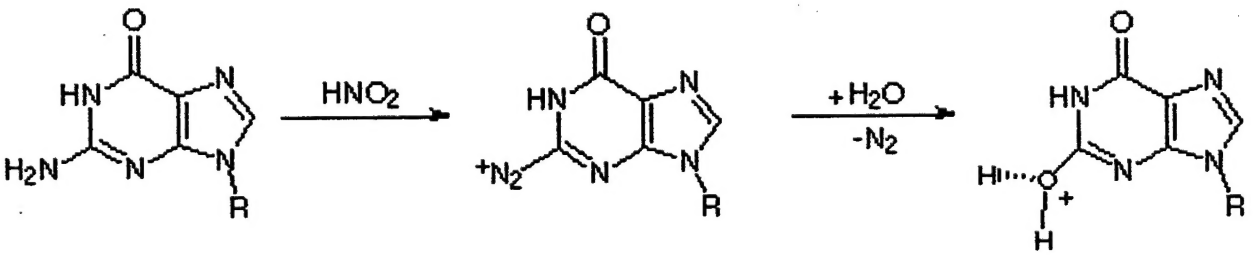
Funded by Life Sciences Undergraduate Research Opportunity Program

Nitrosative deamination of guanine: An ab initio theoretical study of the bimolecular nucleophilic aromatic substitution

Karen Williams, Sundeep Rayat, and Rainer Glaser

The deamination of DNA nucleosides occurs quite readily in the presence of NO, a highly reactive, lipid diffusible species generated intercellularly from the cleavage of the terminal guanidino group of the amino acid L-arginine. NO combines with molecular O_2 generating the species N_2O_3/N_2O_4, which subsequently reacts with the exocyclic amino groups of DNA bases leading to deamination. Prolonged levels of NO are toxic to the body and can lead to acute inflammation, which is thought to be a key role player in certain types of cancers.

It was commonly accepted that the mechanism leading up to the major product of guanine deamination, namely xanthine, followed a simple nucleophilic aromatic substitution pathway with the guaninediazonium ion as the key intermediate. This mechanism assumed that the loss of N_2 and the addition of H_2O occurred with the pyrimidine ring staying intact at all times. However, the product oxanosine was isolated more recently, which could not be explained by the aforementioned mechanism. This problem was eventually solved in 1996 when Glaser et al. showed that the unimolecular mechanism actually occurred via a pyrimidine ring-opened cation. This new mechanism could explain both the major products xanthine and oxanosine.



All studies to date have considered the unimolecular nucleophilic aromatic substitution mechanism. We are now interested in the bimolecular substitution pathways and have been exploring this mechanism theoretically at the B3LYP/6-31G** and MP2(FULL)/6-31G** levels. Thus far we have optimized the diazonium ion and product of the displacement of N_2 by water. It was hypothesized that the structure of this product would be planar. We discovered that the structure of the water adduct was actually pyramidal. We are now determining the structure of the transition state that connects the diazonium ion and the hydrolysis product. Our goal is to discover if the bimolecular pathway also occurs with pyrimidine ring-opening.

Chris Wilson

Hometown: St. Paul, Minnesota

Major: Biology

University: Hamline University

Faculty Mentor: Dr. Shuqun Zhang, Biochemistry

Funded by Plant Genomics Internship at MU

Screening for t-DNA insertional mutants in MAP-kinase pathways in *Arabidopsis thaliana*

Chris Wilson and Shuqun Zhang

The mitogen activated protein kinase (MAP-kinase) pathway is activated in plants under stresses such as pathogen infection or wounding. Its activation leads to many cellular responses including the activation of defense genes and cell death. However, how MAP-kinase activation induces these defense responses is unknown. Studying this pathway will clearly enhance the knowledge of how plants respond to disease and injury. Mutant organisms with disrupted genes in MAP-kinase cascades will be useful tools, because they will have unexpressed or over-expressed genes coding for the enzymes in the MAP-kinase pathway. These mutants will be used to observe changes in phenotype and molecular mechanisms verses wild-type organisms. The University of Wisconsin at Madison has created 72,960 mutant strains of *Arabidopsis thaliana* by inserting t-DNA using random mutagenesis. These plants were grouped into pools, which were then grouped into super-pools. Genomic DNA was prepared from each super-pool. These were used as templates to run PCR with primers from the t-DNA left border and 6 target genes in the MAP -kinase pathway (MPK3, MPK6, MEK4, MEK5, MEK1, and MEK2[3]). Southern blot analysis was used to find super-pools that contained DNA with t-DNA inserted into one of the genes. PCR with a nested primer was used to verify this, and the product was sequenced to see where the t-DNA had inserted in the gene. Two super-pools were found to contain mutants with t-DNA inserted in the coding region of MPK6, and one super-pool was found to have a t-DNA insertion near the promoter region of MEK4.

Dana Lynn Woodruff

Hometown: Kahoka , Missouri

Major: Agronomy

University: University of Missouri-Columbia

Faculty Mentor: Dr. Georgia Davis, Agronomy

Funded by Plant Genomics Internship at MU

Evaluating the Growth of *Aspergillus flavus* with Flavanoids and their Derivatives.

Dana Woodruff and Georgia Davis

Aspergillus flavus is a soil born fungus that infects numerous crop plants such as corn, peanuts, cotton and rice. Aflatoxin is a naturally occurring toxin produced by some strains of the fungus. It is the most potent natural carcinogen found. The U.S. Food and Drug Administration prohibits the sale of grain with aflatoxin levels exceeding 20 parts per billion (ppb). Once corn is found to be contaminated with aflatoxins, very few detoxification and utilization options are available. Some flavanoid compounds and derivatives can alter the growth rate of fungi including *A. flavus* or the amount of toxin produced. In corn a defect in chalcone synthase *(c2)* , a gene controlling the rate-limiting step in anthocyanin (pigment) synthesis, results in a 7-fold increase in toxin production. Our objective is to determine the effect of hesperitin, quercitin, rutin, and naringenin on *A. flavus* growth and aflatoxin production. *A. flavus* strain NRRL3357 was grown on Czepak's media with 10g/L NaCl. The media was supplemented with hesperitin, rutin, naringenin, and quercetin at concentrations of 50mM, 100mM, 150mM, 200mM, and 250mM. The center of each plate was marked and inoculated with a 1 cm square of fungus. Fungal growth was measured from digitalized images at 2, 4, 6, 8, 10, 12, and 14 days after inoculation. At 14 days the fungus was scraped from the plates and weighed for aflatoxin analysis. 14 additional compounds will be analyzed in future experiments. With this information we hope to identify which enzymatic reactions in corn are critical to reducing aflatoxin production and use this information to identify naturally occurring alleles that can be used to produce commercially acceptable corn varieties that inhibit toxin production.